Intra-individual variations and critical differences of clinical laboratory parameters

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Intra - individual variations and critical differences of clinical laboratory parameters

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Promotor: Prof. dr. P.J. Brombacher
Copromoter: Prof. dr. F.W.H.M. Merkus

Referenten: Prof. dr. J.A. Flendrig
Prof. H. de Jonge
Prof. dr. dr. D. Stamm

aan Yolande
aan mijn ouders

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CONTENTS

Chapter 1

Introduction

In medical practice physicians request clinical laboratory tests to assist in making a diagnosis, to suggest a type of treatment and to monitor the patient.
In general, support is sought for the confirmation of a preliminary diagnosis based on the patient's history and physical examination. The latter is often regarded as subject to personal interpretation whilst laboratory tests are considered to provide more objective information. As far as clinical chemistry and haematology are concerned, the information is obtained by chemical and physical procedures, estimating characteristic values of the sample to be analysed and trying to determine these values with the least possible bias.

One example is the determination of the calcium concentration in a blood sample. Different analytical techniques can be used. Although these may lead to slightly different results (depending on the accuracy of the method) the calcium content is constant in the sample. This constancy of the component to be determined is not always present. Protein in a blood sample is by denaturation subject to alteration in time. This implies that the moment of analysing the sample is a factor to be considered in the analytical procedure (1,2). Other factors too can play a role such as a method of sample taking (3,4), whether or not the patient is ambulant or confined to bed, fasting or not fasting, the use of drugs and many other influences (5,6).

Although at first sight these influences may seem of minor importance in the determination of e.g. the element calcium, nevertheless the results of the analyses are certainly dependent on a great number of influences (7).
Amongst these are the mobility of the patient, the time of the day, the way of drawing the blood sample, and in case the amount of ionised calcium has to be determined, also the degree of protein binding. After many investigations the conclusion is generally accepted that determination of components in human blood samples and other samples of human origin to be analysed should be performed under strictly standardised circumstances. Information should be available on at least age of the patient, male/female, drugtherapy, time of taking the blood sample, fasting or not fasting and supine or erect position during blood drawing.

Data obtained by laboratory investigation are used in clinical decision making. Often, laboratory data are reported in numerical form without further information. A more informative lab report might be a great help for the clinician in supporting or rejecting the provisional diagnosis. This extra information is especially helpfull when the difference between health and illness is not evident at first sight. Of vital importance therefore is a survey of test results obtained by the investigation of healthy populations or sub-populations. As a matter of fact, for the diagnosis of a certain disease it is necessary to compare data obtained from the diseased population with those obtained from other populations. The latter may, preferably, consist of healthy individuals although it is not always obvious how to define "healthy" in this context.
Comparison with data from non-healthy individuals, but not having the studied disease, also makes sense. A usual finding is that the ranges of values for the laboratory

determinations from healthy and non-healthy populations and the population of patients with the disease to be studied, overlap to a greater or lesser extent. Decision limits then must be determined. Of course, with decreasing overlap of data the discriminative values of the parameter increases. The commonly used "reference range" is generally determined by analysis of a large number of blood samples from an apparently healthy group of people at a certain time (transverse reference values).
Data obtained from blood samples of patients deviating from this range of "reference values" or "normal values"from the healthy population may indicate a pathological or abnormal situation. These deviating values are often called abnormal values or pathological values. The use of the word "normal" is open for discussion. Many meanings of "normal" can be found in literature. Murphy mentioned in a review paper seven meanings of normal (13).
In statistics "Gaussian distribution" is often called a "normal distribution".
Especially in medicine non-Gaussian distributions are often found.
This is not unexpected, Gauss' law being applied originally to repeated measurements on the same object and not (as is often done) on a series of measurements of different objects or subjects. It sometimes happens that laboratory data obtained from blood samples from healthy individuals can be adequately described by a Gaussian distribution, while another Gaussian distribution (with a different mean) or a non Gaussian distribution is found when blood samples from a diseased population are investigated (8).

The investigated samples from the healthy reference population frequently consist of selected groups such as donors from the blood transfusion service, laboratory personal or medicine students. Evidently, this is not an ideal situation, several selection mechanism's may be present.
When the data can be described according a Gaussian law, the mean value ± twice the standard deviation approximates the so-called "normal range" or better, "reference range", comparising 95% of the data in the population in case the sample size is not too small (at least 100). If a Gaussian distribution is not justified, percentiles are used to derive reference values. Data obtained by analysis of a blood sample presented for investigation are compared to this "reference range".
It is clear that the formulation "normal values" is better substituted by the indication "reference values". The analytical data obtained in the laboratory are compared to the so-called "reference values". The population of patients is sometimes called the target population; the healthy population is the reference population. To be relevant, comparison of patients' values with the reference range assumes three conditions:

1. Biological variability of reference population and target population should be comparable to a large extent; e.g. composition of the groups (males/females) ages of persons in the groups, etc.

2. The analytical data in both populations have to be determined by the same procedures, with similar precision and accuracy.
   If possible, the same apparatus should be used in order to eliminate the influence of the procedure and introduction of a sort of difference which has nothing to do with the blood samples themselves.

3. The pre-analytical treatment of the blood samples to be analysed should be identical both for reference samples and for blood samples from patients.

The foregoing concerns to *transversal* reference data.

Different information is obtained by so-called *longitudinal* investigation (9,10,11,12). In this type of study one or more parameters are determined in consecutive blood samples of a single individual during a longer period.

Longitudinal appreciation has advantages in comparison to the transverse method. Changes in parameter values in a patient can be within the transverse reference range, but, nevertheless, the difference between the values may be very indicative for pathology. E.g. thyroxin values in one patient changing within a certain time from 60 nmol/l to a 128 nmol/l indicate progressive hyperthyroidism, although the transverse reference range is from 55 to 140 nmol/l. Both values of the patient lie within the transverse reference range. However, the change from 60 nmol/l to 128 nmol/l is non-physiological and confirms the diagnosis of hyperthyroidism.
Assessment of repeated or longitudinal data ask for knowledge of intra-individual variability.

A real problem is that sufficient data about intra-individual variation in time are not available for most clinical chemical and haematological parameters. Reliable longitudinal appreciation of data therefore, is in general not possible. The purpose of the present investigation is to assess the intra-individual variations of a number of clinical chemical, haematological and coagulation parameters in a large population of healthy volunteers. Moreover, the so-called critical differences will be considered.

The first part of our study has been concerned with the determination of the intra-individual variance in three different time spans:
- during one day; (4 blood samples of one individual) blood samples were taken during one day from a group of 62 healthy individuals at 8.30 a.m., 11.00 a.m., 2.00 p.m. and 4.30 p.m. These times have been chosen because in normal practice about 95% of blood samples for analysis are drawn within these hours; in this way every volunteer provided four blood samples on that same day;
- during one week; (6 blood samples of one individual) blood samples were taken daily at 9.00 a.m. from all participants of a group of 16 healthy volunteers;
- during six months; (6 blood samples of one individual) blood samples were taken from all participants in a group of 274 healthy volunteers at fixed times for each person, e.g. every first Tuesday of the month at 9.00 a.m.

After analysis of the above mentioned laboratory samples it was possible to calculate from every individual the mean values (from either 4 or 6 data), i.e. each persons own mean values and the corresponding variances (s_T^2). Generally speaking, the variance s_T^2 (total variance) is composed of biological, analytical and "other" variances.
This means, that the total variance s_T^2 for every individual can be calculated by addition of the biological variance s_B^2 and the analytical variance s_A^2 and the "other" variance s_O^2, the total variance thus is concerned with one parameter during a period of either one day or one week or six months and can be written as

$$s_T^2 = s_B^2 + s_A^2 + s_O^2$$

The biological variance s_B^2 is composed generally of an intra-individual variance s_P^2 and an inter-individual variance s_I^2 and consequently can be written as

$$s_B^2 = s_P^2 + s_I^2$$

The analytical variance s_A^2 is composed of the intra-run variance s_S^2 and of the inter-run or long-term variance s_L^2 and can be written as

$$s_A^2 = s_S^2 + s_L^2$$

The intra-run variance s_S^2 for all parameters is determined by analysing in one run a large number (e.g. 36) of blood samples of one physiological human poolserum. This provides mean values $\bar{x}$ and standard deviations for each run and each component. Repeating the procedure several times in the same day with the same serum finally enabled us to calculate mean values for $\bar{x}$ and for the standard deviations. Mean variances for all components could also be calculated from the variance values obtained in each run. These mean variances in our study are designated as s_S^2 *(tables 1.1. - 1.3.)*

The inter-run variances for all parameters have been calculated from daily repeated measurements of components in one freeze-dried human control serum during one year. From all values thus obtained, means values and standard deviations were calculated. The variances have also been calculated for all components and in our study are designated as s_L^2 *(tables 1.1. - 1.3).*
The concentrations of the components in the control sera were within the normal reference range.

It is now possible to give the following formula for the total variance

$$s_T^2 = s_P^2 + s_I^2 + s_S^2 + s_L^2 + s_O^2$$

In the present study we pay special attention to the biological variance s_B^2. Moreover we reduced as far as possible the value of the analytical variance. This could be achieved by analysing all blood samples of one person in the same analytical run whereby the inter-run variance s_L^2 can be omitted and the analytical variance s_A^2 in this case can be written as $s_A^2 = s_S^2$. The so-called "other" variance s_O^2 is mainly dependent of the blood collection and has been greatly reduced by the use of a vacuum blood taking system and standardisation of the procedure. The value of s_O^2 is therefore neglectable in this study.

As had been indicated all variances are determined separately for each individual so that in this study the biological variance is always equal to the intra-individual variance, i.e. $s_B^2 = s_P^2$.
So, finally, the total variance s_T^2 for one individual in this study is now composed of the intra-individual variance s_P^2 and the intra-run variance s_S^2 and can be written as

$$s_T^2 = s_P^2 + s_S^2$$

Assuming the analytical variance to be constant and known. From the latter formula it is possible to calculate the biological variance s_B^2 as follows:

$$s_B^2 = s_P^2 = s_T^2 - s_S^2$$

Table 1.1.
Analytical variations

Analyte	CV_S	CV_L	*Analyte*	CV_S	CV_L
	%	%		%	%
Enzymes			**Intermediary metabolites**		
– CK	1.7	5.1	– Bilirubin	1.6	2.1
– CK-MB	4.3	6.1	– Glucose	1.5	2.1
– ALAT	0.9	1.9	– Cholesterol	1.8	2.9
– ASAT	0.9	2.3	– Triglycerides	2.8	4.6
– LDH	2.1	3.0	– Total protein	1.3	1.6
– α-HBDH	2.8	4.0	– Albumin	1.8	2.4
– γ-GT	1.3	2.5	– Urea	1.8	2.9
– Alk. phos.	0.9	2.2	– Creatinine	1.6	2.6
– α-Amylase	1.2	3.7	– Uric Acid	1.0	3.8
Electrolytes			**Iron status**		
– Sodium	0.6	1.0	– Iron	3.8	4.8
– Potassium	1.0	1.5	– Transferrin	1.9	3.1
– Chloride	1.0	1.5	– Ferritin	10.0	12.4
– Calcium	0.8	2.2			
– Phosphate	1.4	4.1	**Thyroid hormones**		
			– Thyroxin (T_4)	6.4	7.7
			– Thriiodothyronine (T_3)	5.7	6.9

– *CV_S = coefficient of variation within the run*
– *CV_L = coefficient of variation between runs*

In the groups of 62 and 16 and 274 healthy volunteers the intra-individual variances s_P^2 calculated from the values of viz. one day, one week and six months have been determined for each parameter of a group of 55 different laboratory parameters in the fields of clinical chemistry, haematology and coagulation analysis.
The variability of these intra-individual variances can be described in histograms. From these histograms the 50-percentile values and the 90-percentile values of the observed intra-individual variances for every parameters (s_{P50}^2 and s_{P90}^2) have been determined.

In general clinical laboratory practice it is simpler and more common to use coefficient of variations (CV) instead of variances or standard deviations. Especially its biological component,

$$CV_B = \frac{s_B}{\bar{x}} \cdot 100\ \%,$$

calculated for each individual, will be extensively reported.

Table 1.2.
Survey of the analytical parameters, their abbreviations and the analytical variation, determined with the H-6000®.

Analytical parameter	*Abbreviation*	*Analytical variation*	
		CV_S %	CV_L %
Erythrocytic parameters			
Red Blood Cells	RBC	2.7	3.8
Haemoglobin	Hb	2.6	3.6
Haematocrit	Hct	2.9	3.7
Red cell Distribution Width	RDW	1.5	2.1
Thrombocytic parameters			
Platelets	Plt	7.0	8.1
Mean Platelet Volume	MPV	3.0	3.8
Platelet Distribution Width	PDW	3.4	4.0
Plateletcrit	Pct	6.0	6.9
Leucocyte and Differential Count			
White Blood Cells (total)	WBC	7.0	7.9
Neutrophils		1.4	2.0
Lymphocytes		1.8	2.3
Monocytes		6.6	7.8
Eosinophils		7.5	8.6
Basophils		9.7	11.3
Large Unstained Cells	LUC	13.6	15.4
High Peroxidase Cells	HPX	23.2	28.1

The second part of our study will been concerned with the determination of critical differences (d_K).
The meaning of the critical difference d_K is to indicate the range which covers the great majority (95%) of the differences between two obtained values of one laboratory parameter in one individual.
The critical difference is dependent on the total variance s_T^2 for the one individual concerned. Therefore it is dependent on the biological, analytical and "other" variances.
Young and Stamm (9,10) proposed to consider for the critical difference two times the standard deviation of the difference of two observations. This leads to the following formula for the calculation

$$d_K = 2\sqrt{2 s_T^2}$$

Table 1.3.
Analytical variations

Analyte	CV_S %	CV_L %
Normotest	5.3	6.1
Thrombotest	6.3	7.0
Calcium thromboplastin time	3.4	4.0
Activated partial thromboplastin time	3.3	4.0
Thrombin time	5.0	6.5
Fibrinogen	3.1	3.9
Factor V	3.5	4.1
Antithrombin III	2.1	2.5
α_2-Antiplasmin	2.0	2.3
Plasminogen	2.0	2.4
Factor X	2.2	2.9

The choice of the factor 2 of course is arbitrary but based on the wanted 95% probability.

In our study the biological variance equals the intra-individual variance ($s_B^2 = s_P^2$) as had been indicated before. Also, the analytical variance equals the intra-run variance ($s_A^2 = s_S^2$) and the "other" variance s_O^2 can be neglected being small and constant.
It follows that in our study the critical difference is predominantly dependent on the intra-individual variance s_P^2 and the intra-run variance s_S^2 and can be written as

$$d_K = 2\sqrt{2\,s_T^2} = 2\sqrt{2\,(s_P^2 + s_S^2)}.$$

When longitudinal investigations are performed in clinical (laboratory) practice, it is not possible to restrict the analytical variance s_A^2 to the intra-run variance s_S^2, as patient's samples generally will not be stored untill the end of the study. The inter-run variance s_L^2 then has to be taken into consideration and the calculation of the critical difference will be as follows:

$$d_K = 2\sqrt{2\,(s_P^2 + s_L^2)}.$$

The so calculated critical differences will be larger than those in our study. These critical differences d_K are expressed in relevant units because they have been calculated from variances. It is of course possible to express the critical differences in percentages through the use of coefficient of variation.

References

1. **Wisser H. and Knoll E.**
Ärztl. Lab. 1982; 28: 99-108

2. **Simpson H.W.**
Essays Med. Biochem. 1976; 2: 115-187

3. **Falch D.K.**
Scand. J. Clin. Lab. Invest. 1981; 41: 59-65

4. **Kupke I.R., Kather B. and Zeugner S.**
Clin. Chim. Acta 1981; 112: 177-185

5. **Schwartz M.K.**
Adv. Clin. Chem. 1972; 16: 1-45

6. **Young D.S.**
Biological variability. In: Brown S.S., Mitchel F.L. and Young D.S.,ed. Chemical diagnosis of disease. Amsterdam: Elsevier/North-Holland, 1979; 3-95

7. **Kreutzer H.H. and Raymakers J.A.**
Interpretatie van uitkomsten van laboratoriumonderzoek in de geneeskunde. Utrecht/Antwerpen: Bohn, Scheltema & Holkema, 1982; 1-140

8. **Elveback L.R., Guiller C.L. and Keating F.R. Jr.**
JAMA 1970; 211: 69-75

9. **Young D.S.**
Clin. Chem. 1978; 24: 212-222

10. **Stamm D.**
Clin. Chem. Clin. Biochem. 1982; 20: 817-824

11. **Statland B.E. and Winkel P.**
CRC Crit. Rev. Clin. Lab. Sci. 1977; 10: 105-144

12. **Lommel H. and Weyer M.G.**
Lab. Med. 1982; 6: 14-21

13. **Murphy E.A.**
Perspect. Biol. Med. 1972; 15: 566-582

Chapter 2

Intra-individual variations and calculated critical differences of clinical laboratory parameters during one day

2.1. Introduction

It has been pointed out in the first chapter that the variability of laboratory values in the course of one day would be studied. Here this part of our investigations is described. In an introductory paragraph the general information concerning participants, materials and methods and variance components is given.
Then three paragraphs follow describing the results obtained in viz. clinical chemistry, haematology and laboratory study of coagulation.

Individuals

The investigation has been started with a group of 62 volunteers who were free of complaints concerning their health in the age of 18 - 53 years old. The groups contained 23 males and 39 females. Some were hospital laboratory personal (20 persons), clinical personal (16) and students (26 persons). By anamnesis volunteers with history of disease within the last 3 months and/or current diseases were excluded.
The volunteers length, weight, age and sex were registered. Smoking and the use of oral contraceptives were no exclusion criteria.

Materials and methods

Specimens:
All blood specimens from one individual were taken at fixed times i.e. 8.30 and 11.00 a.m. and 2.00 and 4.30 p.m., in the course of one day. The individuals had a 10 to 15 minutes rest in a sitting position before venipuncture. While the subject was in a comfortable sitting posture blood was collected in four evacuated blood collection tubes. (Terumo ®). Brief tourniquet pressure was released immediately on venipuncture.
For the clinical chemical analyses the vacuum tubes contained no additive. The analyses have been performed in serum. For haematology the tubes contained K-EDTA as an anti-coagulant and for coagulation analyses the tubes contained sodium-citrate as an anti-coagulant.

Analytical procedures:
The analytical procedures used for the clinical chemical and coagulation parameters were the same as used in daily routine for analysis of patients' specimens *(tables 2.1.1. and 2.1.2.)*.
The automated analyser used for the haematological parameters was the same as used in daily routine for analysis of patients' specimens (H-6000®, Technicon Instruments Corporation, Tarrytown, New York).

Tabel 2.1.1.
Survey of methods and apparatus

Analyte	*Method*	*Apparatus*	*Lit.*
Enzymes			
– CK	DGKC (37°C)	CFA	1
– CK-MB	DGKC (37°C) Inhibition of the M-fraction	CFA	2-3
– ALAT	SCE	CFA	4
– ASAT	SCE	CFA	4
– LDH	SCE	CFA	4
– α-HBDH	DGKC (37°C)	CFA	5-6
– γ-GT	L-γ-glutamyl-3-carboxy-4-nitro-anilide	CFA	7-8
– Alkaline phosphatase	Hydrolysis of p-nitrophenyl-phosphate in AMP-buffer (37°C)	CFA	9
– α-Amylase	Enzymatic colorreaction with p-nitrophenylmalthoheptaoside as a substrate	CFA	10
Electrolytes			
– Sodium	Flame photometry after dialysis	Flame photometer	11
– Potassium	Flame photometry after dialysis	Eel Corning	11
– Chloride	Argentometry	Chlorocounter	12
– Calcium	Titrimetry/Fluorimetry	Eel Corning	13
– Phosphate	Phosphomolybdate formation measured at 350 nm	CFA	14
Intermediary metabolites			
– Bilirubin	Jendrassik-Grof	CFA	15
– Glucose	Hexokinase/G6P-DH	CFA	16-17
– Cholesterol	CHOD-PAP	CFA	18-19
– Triglycerides	Fully enzymatic measurement without correction of free glycerol	CFA	20
– Total protein	Biuret reaction with serum blank	CFA	21-22
– Albumin	Bromcresol purple method	CFA	23
– Urea	Urease	CFA	24
– Creatinine	Jaffé-reaction	CFA	25-26
– Uric Acid	Uricase measurement	CFA	27-28
Iron status			
– Iron	Bathophenantroline in not deproteinated serum with serum blank	CFA	29
– Transferrin	Immunological, turbidimetric meas.	CFA	30-33
– Ferritin	Immunophase ™ Ferritin (^{125}J)	IRMA technique	34-35

Analyte	*Method*	*Apparatus*	*Lit.*
Thyroid hormones			
– Thyroxin (T_4)	Autopak T_4 RIA	Micro Med. Syst.	36-38
– Triiodothyronine (T_3)	Autopak T_3 RIA	Micro Med. Syst.	36-37

– CFA = Centrifugal Fast Analyser (Cobas Bio^R of Hoffman-La Roche, Basel)

Tabel 2.1.2.
Survey of methods and apparatus

Analyte	*Method*	*Apparatus*	*Lit.*
Normotest	According to Owren	manual	39
Thrombotest	According to Owren	Lode	40
Calcium thrombo-plastin time	According to Quick (human-brain thromboplastin)	Schnittger & Gross	41
Activated partial thromboplastin time	Thromboplastin and actin as contact activator	Schnittger & Gross	42
Thrombin time	Limited thrombin concentration as coagulation-activator	Schnittger & Gross	43
Fibrinogen	According to Claus	Schnittger & Gross	44
Factor V	According to Stormorken	Schnittger & Gross	45
Antithrombin III	Amidolytic (S 2238)	CFA	46
α_2-Antiplasmin	Amidolytic (S 2251) and streptokinase as activator	CFA	47
Plasminogen	Amidolytic (S 2251)	CFA	48
Factor X	Amidolytic (S 2337)	CFA	49

– Lode = coagulation analyser of Lode, Groningen
– Schnittger & Gross = coagulation analyser of Salm & Kipp, Breukelen
– CFA = Centrifugal fast analyser (Cobas Bio^R of Hoffman-La Roche Basel)

Variance components:
Broadly speaking three components can be distinghuished a biological one, an analytical one and an "other" one, see table 2.1.3. for the symbols according to Stamm (50)
For the present investigation the "other" component can be neglected, specimen collection is usually its mean part and to minimise this component, in this study the collection is standardised and moreover a vacuum collecting system is used.
For each individual total intra-individual variances (s_T^2) can be computed from the observations. Assuming the analytical component to be known,the biological one can be computed from
$s_B^2 = s_T^2 - s_A^2$.
In this study we only consider intra-individual variation (so s_I is absent) and all samples from one individual were analysed in the same run (so s_L is absent). According the

terminology from *table 2.1.3.* we have $s_B = s_P$ and $s_A = s_S$. (see also chapter 1).
The extra index "day", which may be justified in this study for all components is omitted.
Reported results were mainly given in coefficients of variation (percentages) for instance

$$CV_B = \frac{s_B}{\bar{x}} \times 100\ \%,$$

whereas $\bar{x}$ is based on the observations of one individual.

Table 2.1.3.
Symbols for the variance components used in this study (50)

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s_T^2 = total variance of one individual from a reference population.

$$s_T^2 = s_B^2 + s_A^2 + s_O^2$$

s_B^2 = biological variance

$s_B^2 = s_P^2 + s_I^2$ $\quad$ s_P^2 = intra-individual variance
s_I^2 = inter-individual variance
s_I^2 absent in this study

s_A^2 = analytical variance

$s_A^2 = s_S^2 + s_L^2$ $\quad$ s_S^2 = variance within the run
s_L^2 = variance between runs
s_L^2 absent in this study

s_O^2 = "other" variance, e.g. specimen collection

Corresponding coefficient of variation (percentages) are denoted by CV_T, CV_B and CV_A.

d_K =critical difference

$$d_K = 2\sqrt{2s_T^2} = 2\sqrt{2(s_P^2 + s_S^2)}$$

or

$$d_K = 2\sqrt{2CV_T^2} = 2\sqrt{2(CV_P^2 + CV_S^2)}$$

To characterise the variability of CV_P for each parameter, three characteristics of the histogram of 62 CV_P values will be reported in the next tables:
- the percentage of individuals with $CV_T > CV_S$ (denoted by n_{var}).
- the median, denoted by CV_{P50}
- the ninethy percentile, denoted by CV_{P90}

Critical difference:
The critical difference d_K intends to be a tool to evaluate the difference between two or more obtained values of one laboratory parameter in one individual.
The critical difference is dependent on the total variance s_T^2 for the one individual concerned. (see also chapter 1).
It follows that in our study the critical difference is predominantly dependent on the intra-individual variance s_P^2 and the within run variance s_S^2 can be written as:

$$d_K = 2\sqrt{2\,s_T^2} = 2\sqrt{2\,(s_P^2 + s_S^2)} \quad \text{(in units)}.$$

or

$$d_K = 2\sqrt{2\,CV_P^2 + CV_S^2} \quad \text{(in percentages)}.$$

When longitudinal investigations are performed in clinical laboratory practice, the analytical variance s_A^2 is composed of the intra-run variance s_S^2 and of the inter-run variance s_L^2: the calculation of the critical difference will be as follow:

$$d_K = 2\sqrt{2\,(s_P^2 + s_L^2)} \quad \text{(in units)}.$$

or

$$d_K = 2\sqrt{(2\,CV_P^2 + CV_L^2)} \quad \text{(in percentages)}$$

Statistical methods:
For each clinical parameter it has been investigated with the Friedman rank test (51) whether a systematic pattern existed. Such a pattern could be an upward or a downward trend or a systematic low or high value at one particular time point.
Several two group comparisons (like male versus female) were performed using the Mann-Whitney test (52). Correlations were studied using the Spearman rank correlation test (52).

References

1. **Recommandations of the German Society of Clinical Chemistry**
   J. Clin. Chem. Clin. Biochem. 1977; 15: 255

2. **Würzburg U. et. al.**
   Klin. Wschr. 1976; 53: 357

3. **Würzburg U. et. al.**
   J. Clin. Chem. Clin. Biochem. 1977; 15: 131

4. **The Comittee of Enzymes of the Scandinavian Society for Clinical Chemistry and Clinical Physiology.**
   Scand. J. Clin. Lab. Invest. 1974; 33: 287

5. **Recommandations of the German Society of Clinical Chemistry**
   J. Clin. Chem. Clin. Biochem. 1970; 8: 658

6. **Recommandations of the German Society of Clinical Chemistry**
   J. Clin. Chem. Clin. Biochem. 1972; 10: 182

7. **Persijn J.P. and van der Silk W.**
   J.Clin. Chem. Clin. Biochem. 1976; 14: 421

8. **Szasz G. et. al.**
   Z. Klin. Chem. Klin. Biochem. 1974; 12: 228

9. **Nederlandse Vereniging voor Klinische Chemie**
   Mededelingenblad 1979 4: 314

10. **Raucher E. et. al.**
    Ber. Österr. Ges. Klin. Chem. 1981; 4: 150

11. **Hald P.M.**
    J. Biol. Chem. 1947; 167: 499

12. **920 Chloride-Meter**
    Corning Medical, Corning Limited, Halstead Essex C092DX England

13. **Diehl H. and Ellingbase P.**
    J. Anal. Chem. 1956; 28: 882

14. **Amador E. and Urban J.**
    Clin. Chem. 1972; 18: 601

15. **Jendrassik L. and Grof P.**
    Biochem. J. 1938; 297: 81

16. **Massod M.F.**
    Am. J. Med. Technol. 1976; 43: 243

17. **Schmidt F.H.**
    Klin. Wschr. 1961; 39: 1244

18. **Röschlau P.E. et. al.**
    9th. Int. Congr. on Clin. Chem. Toronto 1975. Abstr. no. I

19. **Trinder P.**
    Ann. Clin. Biochem. 1969; 6: 24

20. **Bucolo G. and David H.**
    Clin. Chem. 1973; 19: 476

21. **Weichselbaum T.E.**
Am. J. Clin. Pathol. 1946; 16: 40

22. **Eisenweiner H.G.**
Med. Lab. 1975; 28: 27

23. **Pinell A.E. and Northam B.E.**
Clin. Chem. 1978; 24: 80-86

24. **Tiffany T.O. et. al.**
Clin. Chem. 1972; 18: 1892

25. **Fabing D.L. and Ertingshausen G.**
Clin. Chem. 1971; 17: 696

26. **Bartels H. and Böhmer M.**
Clin. Chim. Acta 1971; 32: 81-86

27. **Practorius E. and Poulsen H.**
Scand. J. Clin. Lab. Invest. 1953; 5: 273

28. **Mahler J.L.**
Anal.Biochem. 1970; 38: 165

29. **Giovanniello T.L. and Peter T. Jr.**
Iron-method. In: Seligson D., ed. Standard Methods of Clinical Chemistry. New York: Academic Press, 1963; 139

30. **Wider G. et. al.**
Ber. Österr. Ges. Klin. Chem. 1981; 4: 33

31. **Weippl G.**
Blut 1973; 27: 376

32. **Neumann U. et. al.**
Lab. Med. 1978; 2: 62

33. **Neumann U. et. al.**
J. Biol. Stand. 1978; 6: 133

34. **Addison G.M. et. al.**
J. Clin. Pathol. 1972; 25: 326

35. **Miles L.E.M. et. al.**
Anal. Chem. 1974; 61: 209

36. **Yalon R.S. and Berson S.A.**
J. Clin. Invest. 1960; 39: 1157

37. **Shelley D.S. et. al.**
Clin. Chem. 1973; 19: 146

38. **Chopra I.J.**
J. Clin. Endocrinol.Metab. 1972; 34: 938

39. **Owren P.A.**
Human blood coagulation. In: Hemker H.C., Loeliger E.A. and Veltkamp J.J., ed Biochemistry clinical investigation and therapy. Leiden: University Press, 1969; 369-388

40. **Owren P.A.**
Lancet 1954, 2: 754

41. **Biggs R and MacFarlane R.G.**
Human blood Coagulation and its Disorders.
Oxford: Blackwell Sc. Publications, 1962

42. **Langdell R.D. et al.**
J. Lab. Clin. Med. 1953: 41: 637

43. **Nilsson I.M.**
Hemorrhagic and Thrombotic Diseases. London: John Wiley & Sons, 1974

44. **Claus A.**
Acta Haemat. 1957; 17: 237

45. **Stormorken H.**
Thrombos. Diathes. haemorrh. 1960; 5: 267

46. **Abilgaard II et al.**
Thromb. Res. 1977; 2: 549

47. **Matter L.E. et al.**
Thromb. Haemostas. 1977; 38: 776

48. **Teger-Nillson A.C. et al.**
Lab. Invest. 1977; 37: 403

49. **Aurell L.**
Thromb. Res. 1977; 11: 595

50. **Stamm D.**
Control of Analytical Variation in the Production of Reference Values. In: Gräsbeck R. and Alström T., ed. Reference Values in Laboratory Medicine. New York: John Wiley & Sons, 1981; 109-126

51. **Friedman M.**
J. Am. Statist. Assoc. 1937;32: 675-701

52. **Armitage P.**
Statistical Methods in Medical Research. Oxford: Blackwell Sc. Publications, 1971

2.2. Clinical chemical parameters

Introduction

Laboratory parameters are often used in the study of the course of a pathological process, e.g. during treatment of a disease. Therefore, proper interpretation of laboratory data is important.
Interpretation of a single observation has to be done using so called references values (1,2,3), which are based on inter-individual variation. Interpretation of longitudinal observations should be based on intra-individual variation. The present paper assesses the intra-individual variation within one day. Its results can be used for the interpretation of an observation repeated within one day. Blood specimens taken from one individual at different times of one day (i.e. 8.30 and 11.00 a.m. and 2.00 and 4.30 p.m.) shows of course variability.

The variation within one day has three components: a biological one, an analytical one and an "other" component (4), see also chapter 1.
For the interpretation of a patients observation the mentioned variation components should be known in order to detect pathological variation, i.e. variation within one day which exceeds the usual biological and analytical variations.

Using the biological and the analytical variation the so called critical differences can be computed (5,6,7)(see also chapter 1). They give the range for the difference between two repeated measurements within one day, which can be expected in the great majority of "reference" (or "normal") individuals.

In the present investigation a number of 28 clinical chemical blood parameters have been studied in a group of 62 healthy volunteers. Blood specimens were taken at consecutive fixed times within one day for each volunteer in order to assess the intra-individual variations during that day.

Assuming the analytical variation components to be known the biological ones were computed. Moreover, critical differences have been determined.

Results and discussion

The Friedman rank (8) test was applied to the obtained data in order to investigate whether some systematic pattern existed in the consecutive parameter values during the day. No such patterns have been observed, except slight trends for CK and ASAT (increase during the day) and serum bilirubin concentration (decrease during the day).

In blood samples taken at different times of one day, intra-individual variations, not only due to the analytical variability, were calculated. Two situations can occure either $CV_T > CV_S$ in which case positive CV_P is found or $CV_T \leq CV_S$ in which case CV_P is set to zero. The first situation has been observed for the great majority of the individuals for most parameters. For 20 out of the 28 parameters show more than 85% of the participating individuals a positive CV_P. The results are summarised in *table 2.2.1.*

Table 2.2.1.
Intra-individual variation during one day (n=62)

Analyte	n_{var}	CV_S	CV_{P50}	d_{K50}	CV_{P90}	d_{K90}
	%	%	%	%	%	%
Enzymes						
– CK	96	1.7	8.3	24.0	18.5	52.5
– CK-MB	95	4.3	13.5	40.1	25.1	72.0
– ALAT	86	0.9	5.3	15.2	10.3	29.2
– ASAT	95	0.9	4.7	13.5	9.4	26.7
– LDH	89	2.1	5.0	15.3	9.7	28.1
– α-HBDH	79	2.8	4.3	14.5	10.0	29.4
– γ-GT	96	1.3	5.7	16.5	12.8	36.4
– Alk. phos.	94	0.9	2.7	8.0	5.1	14.6
– α-Amylase	100	1.2	4.4	12.9	9.4	26.8
Electrolytes						
– Sodium	73	0.6	0.6	2.4	1.4	4.3
– Potassium	100	1.0	4.6	13.3	7.8	22.2
– Chloride	86	1.0	1.1	4.2	2.4	7.4
– Calcium	79	0.8	1.8	5.6	3.6	10.4
– Phosphate	96	1.4	10.4	29.7	19.6	55.6
Intermediary metabolites						
– Bilirubin	100	1.6	13.4	38.2	24.9	70.6
– Glucose	100	1.5	13.3	37.9	25.8	73.1
– Cholesterol	87	1.8	2.8	9.4	6.4	18.8
– Triglycerides	100	2.8	14.3	41.2	33.9	96.2
– Total protein	92	1.3	2.3	7.5	4.7	13.8
– Albumin	81	1.8	2.4	8.5	5.0	15.0
– Urea	95	1.8	4.8	14.5	9.7	27.9
– Creatinine	94	1.6	3.4	10.6	6.8	19.8
– Uric Acid	94	1.0	5.4	15.5	9.8	27.9
Iron status						
– Iron	89	3.8	9.4	28.7	22.3	64.0
– Transferrin	84	1.9	3.0	10.0	5.7	17.0
– Ferritin	25	10.0	0.0	28.2	11.4	42.9
Thyroid hormones						
– Thyroxin (T_4)	30	6.0	0.0	17.0	6.0	24.0
– Triiodothyroxine (T_3)	51	5.7	0.4	16.1	10.4	33.5

– n_{var} = *percentage of individuals with* $CV_T > CV_S$
– CV_{P50} = *median intra-individual coefficient of variation*
– CV_{P90} = *90 percentile of intra-individual coefficient of variation*

– d_{k50} = *critical difference based on* CV_{P50}
– d_{K90} = *critical difference based on* CV_{P90}
– CV_T = *total coefficient of variation of one individual from a reference population*
– CV_S = *intra-run coefficient of variation*

The within day intra-individual variations, CV_{P50} and CV_{P90} were allmost all greater than zero for the serum constituents except for the CV_{P50} of ferritin and thyroxin. As shown in *table 2.2.1.* the intra-individual variations varied from 0.0 - 14.3% (CV_{P50}) and from 1.4 - 33.9% (CV_{P90}). The critical differences varied from 2.4 - 41.2 (d_{k50}) and from 4.3 - 96.2 (d_{k90}).

Using the Mann-Whitney (9) test it has been investigated for which parameters differences in biological variations (CV_P) between the 50 percentile values and the 90 percentile values for male/female groups could be found. The same was investigated for male smokers/non smokers group, the female smokers/non smokers groups and the female groups using/not using oral contraceptives.

It appeared that only significant differences existed between the male versus the female groups for serum transferrin and triglycerides, both seems to be also clinically important *(table 2.2.2.)*

Table 2.2.2.
Significant differences in the intra-individual variations during one day between males and females ($P < 0.05$)

male/female	n	CV_S %	CV_{P50} %	d_{K50} %	CV_{P90} %	d_{K90} %
Triglycerides						
– males	23	2.8	17.1	49.0	38.1	108.1
– females	39	2.8	11.8	34.3	28.8	81.8
Transferrin						
– males	23	1.9	1.8	7.4	4.8	14.6
– females	39	1.9	3.9	12.3	7.0	20.5

– *n = number of persons*

The intra-individual variation caused by the use of oral contraceptives is given in *table 2.2.3.* The observed differences, ASAT, sodium and thyroxin, seem to be of clinical relevance only for ASAT and sodium. The influence of smoking on the intra-individual variation of the parameters is shown in *table 2.2.4.*
It can be seen, that there are significant differences for the intra-individual variations of potassium, creatinine and ferritin in males. In our opinion only the influences on the creatinine and ferritin intra-individual variations are of clinical importance. A statistically significant influence of smoking versus non-smoking in females was observed for the intra-individual variation of calcium.

Table 2.2.3.
Significant differences in the intra-individual variations during one day between females using or not using oral contraceptives (P <0.05)

female using/not using oral contraceptives	n	CV_S %	CV_{P50} %	d_{k50} %	CV_{P90} %	d_{k90} %
ASAT						
– using	16	0.9	6.2	17.7	13.1	37.1
– not using	21	0.9	4.2	12.1	9.4	26.7
Sodium						
– using	16	0.6	1.1	3.5	3.2	9.2
– not using	21	0.6	0.3	1.9	0.9	3.1
Thyroxin (T_4)						
– using	16	6.0	0.0	17.0	6.7	25.4
– not using	21	6.0	0.0	17.0	7.2	26.5

Table 2.2.4.
Significant differences in the intra-individual variations during one day between smokers and non smokers (P <0.05)

male	n	CV_S %	CV_{P50} %	d_{K50} %	CV_{P90} %	d_{K90} %
Potassium						
– smokers	7	1.0	6.4	18.3	7.5	21.4
– non smokers	16	1.0	4.7	13.6	7.6	21.7
Creatinine						
– smokers	7	1.6	2.7	8.9	4.4	13.2
– non smokers	16	1.6	4.4	13.2	8.3	23.9
Ferritin						
– smokers	7	10.0	0.0	28.3	20.5	64.5
– non smokers	16	10.0	0.0	28.3	2.7	29.3
female						
Calcium						
– smokers	8	0.8	1.7	5.3	2.1	6.4
– non smokers	31	0.8	1.9	5.8	3.8	11.0

In *table 2.2.5.* are summarised the critical differences based on s_L for both males and females, expressed as absolute values in SI units. The use of d_{K50} or d_{K90} is arbitrary. However, for clinical decisions d_{K90} is preferable for maximum specifity, whereas greater sensitivity is obtained using d_{k50}.

Table 2.2.5.
Critical difference on one day based on s_L

Analyte		*mean values*	d_{K50}	d_{K90}	*transverse reference values*
		S.I. units			
Enzymes					
– CK	U/L	103	28	56	< 240
– CK-MB	U/L	10	4	7	< 25
– ALAT	U/L	13	2	4	< 35
– ASAT	U/L	19	3	5	< 30
– LDH	U/L	281	46	81	< 450
– α-HBDH	U/L	124	21	38	90-180
– γ-GT	U/L	16	3	6	4-50
– Alkaline phosphatase	U/L	72	7	11	50-125
– α-Amylase	U/L	117	19	33	70-300
Electrolytes					
– Sodium	mmol/l	144	5	7	133-145
– Potassium	mmol/l	4.3	0.6	1.0	3.5-5.1
– Chloride	mmol/l	107	6	9	95-107
– Calcium	mmol/l	2.5	0.2	0.3	2.2-2.6
– Phosphate	mmol/l	1.1	0.3	0.6	0.9-1.5
Intermediary metabolites					
– Bilirubin	μmol/l	8.3	3.2	5.9	3.4-17.0
– Glucose	mmol/l	4.4	1.7	3.2	3.5-5.6
– Cholesterol	mmol/l	4.6	0.5	0.9	4.0-7.5
– Triglycerides	mmol/l	1.17	0.50	1.13	0.85-2.00
– Total protein	g/l	73	6	10	65-80
– Albumin	g/l	48	5	8	35-50
– Urea	mmol/l	4.5	0.7	1.3	3.0-7.0
– Creatinine	μmol/l	86	10	18	45-130
– Uric Acid	mmol/l	0.26	0.05	0.08	0.16-0.43
Iron status					
– Iron	μmol/l	19	6	12	10-30
– Transferrin	g/l	3.1	0.4	0.6	2.0-3.7
– Ferritin	ng/ml	66	23	31	12-302
Thyroid hormones					
– Thyroxin (T_4)	nmol/l	109	24	30	60-150
– Triiodothyronine (T_3)	nmol/l	2.4	0.5	0.8	1.3-3.0

– *Mean values are taken over all time points and all individuals*

References

1. **Amador E.**
   JAMA 1975; 232: 953-955

2. **Martin H.F., Gudzinowicz B.J. and Fanger H.**
   Normal Values in Clinical Chemistry: A Guide to Statistical Analysis of Laboratory Data. New York: Marcel Dekker, 1975; 1-454

3. **Winkel P. and Statland B.E.**
   Reference Values. In: Henry J.B., ed. Clinical Diagnosis and Management by Laboratory Methods. 16th. ed. Philadelphia:W.B. Saunders, 1979; 29-52

4. **Statland B.E. and Winkel P.**
   CRC Crit. Rev. Clin. Lab. Sci. 1977; 10: 105-144

5. **Young D.S.**
   Clin. Chem. 1978; 24: 212-222

6. **Stamm D.**
   Control of Analytical Variation in the Production of Reference Values. In: Gräsbeck R. and Alström T., ed. Reference Values in Laboratory Medicine. New York: John Wiley & Sons, 1981; 109-126

7. **Costongs G.M.P.J., Janson P.C.W. and Brombacher P.J.**
   J. Clin. Chem. Clin. Biochem., submitted for publication. 1984.

8. **Friedman M.**
   J. Am. Statist. Assoc. 1937; 32: 675-701

9. **Armitage P.**
   Statistical Methods in Medical Research. Oxford: Blackwell Sc. Publications, 1971.

2.3. Haematological parameters

Introduction

In the past the quantitation of haematological parameters has been rather difficult because of the imprecision of the analytical procedures, which were mainly manualeye counting one's.

The recent introduction of a new precise automated analyser (H-6000®, Technicon Instruments Corporation, Tarrytown), combining the quantitation of the complete blood cell count with the determination of the differential leucocyte count, has now decreased the analytical variation. Especially for the differential count the analytical variations have become smaller, mainly due to the classification of about 10,000 leucocytes in each analysis (1,2,3,4)

Moreover, the new developed haematology analyser has made it possible to determine a series of other partly new parameters in the haematological field. The leucocytes are classified according to size and peroxidase activity, except for the basophils. The basophils are separated from the rest of the cells on the basis of a colorimetric reaction with the heparin of the basophils.
A survey of the parameters analysed and the analytical precision obtained with the H-6000® has been given in *table 1.2.*

In this study we calculated the within-day physiological intra-individual variation. The within-day variation exists of different components e.g. a biological and an analytical one. For the interpretation of the data of a patient's specimen analysis the mentioned variation components should be known in order to detect pathological variation, i.e. variation within one day which exceeds the usual biological and analytical variations. Using the biological and the analytical variations, the so called critical differences can be calculated (5,6,7)

In the present study the complete blood cell count, the white cell differential count and some new parameters (RDW, PDW, MPV, Pct, LUC, HPX) have been investigated in a group of 62 apparently healthy volunteers.

Results and discussion

Applying the Friedman rank test (9) to the patient's haematology results there could not be shown any systematic low or high value at an one single time point of the four considered time points.
However there was seen a significant ($p < 0.05$) upward (in the course of the day) trend for the platelet count, mean platelet volume, plateletcrit, the leucocyte count and the percentage of high peroxidase cells *(table 2.3.1.).* No downward trends over the day have been seen. The observed changes, possibly can be a result of the increasing physiologic stress in the course of the day. This stress urges the bone marrow to produce in a higher rate platelets and leucocytes.
The formed cells are the "younger" one's of which the platelets distinct themselves from the "older" one's by volume (increased mean platelet volume and therefrom the plateletcrit becomes higher), whereas the younger leucocytes contain a higher peroxidase activity than the older one's.

Table 2.3.1.
Results of the Friedman rank test for the complete blood cell count and the differential count, in which a significant trend over the four vena puncture times was found

Parameter	*Mean values*				*P-values*
Mean ranks	*8.30 a.m.*	*11.00 a.m.*	*2.00 p.m.*	*4.30 p.m.*	
Platelets	2.08	2.13	2.78	3.06	0.001
Mean Platelet Volume	2.17	2.32	2.52	2.98	0.004
Plateletcrit	2.07	2.12	2.78	3.06	0.001
Total leucocytes	1.72	2.35	2.57	3.36	0.001
High Peroxidase Cells	1.93	2.32	2.85	2.90	0.001

In the blood samples taken at different fixed times of one day, only in a minority (6/16) of the parameters a variation was shown. In that case more than 85% of the participating individuals show a variation *(table 2.3.2.)*. The variability of the studied parameters – as shown in *table 2.3.2.* – is expressed as n_{var} (i.e. the percentage of the variable individuals in the group per parameter).

Table 2.3.2.
Classification of the haematological parameters investigated according to their variability

Variability	*Parameters*
Least variability ($n_{var} = 0 - 50\%$)	Hct, RDW, Plt, MPV, PDW
Intermediate variability ($n_{var} = 51 - 85\%$)	RBC, Hb, Pct, WBC, HPX
Greatest variability ($n_{var} = 86 - 100\%$)	neutrophils, lymphocytes, monocytes eosinophils, basophils, LUC

This variability can best be observed for those parameters with $CV_S < CV_T$. The precision of the analytical method therefore is very important. The results of the analytical precision have been shown in *table 1.2.*. *Table 2.3.3.* contains the computed results for CV_{P50}, d_{K50}, CV_{P90} and d_{K90}, as calculated from the parameters for each individual. The d_{K50} varied from 4.2 - 74.4%, whereas the d_{K90}, showed a range from 6.4 - 146.5

Table 2.3.3.
Intra-individual variation during one day (n=62)

Parameters	n_{var} %	CV_S %	CV_{P50} %	d_{K50} %	CV_{P90} %	d_{K90} %
Erythrocytic parameters						
Erythrocytes	52	2.7	0.6	7.8	4.4	14.6
Haemoglobin	58	2.6	1.1	8.0	4.3	14.2
Haematocrit	45	2.9	0.0	8.2	4.6	15.4
Red cell Distribution Width	47	1.5	0.0	4.2	1.7	6.4
Thrombocytic parameters						
Platelets	38	7.0	0.0	19.8	6.7	27.4
Mean Platelet Volume	32	3.0	0.0	8.5	3.8	13.7
Platelet Distribution Width	27	3.4	0.0	9.6	3.8	14.4
Plateletcrit	58	6.0	2.7	18.6	13.2	41.0
Leucocyte and Differential Count						
Total leucocytes	75	7.0	9.5	33.4	19.9	59.7
Neutrophils	98	1.4	6.5	18.8	15.1	42.9
Lymphocytes	100	1.8	10.0	28.7	23.6	66.9
Monocytes	88	6.6	11.3	37.0	24.8	72.6
Eosinophils	88	7.5	16.3	50.7	28.8	84.2
Basophils	88	9.7	19.4	61.3	41.3	120.0
Large Unstained Cells	80	13.6	14.5	56.2	32.7	100.2
High Peroxidase Cells	55	23.2	12.4	74.4	46.3	146.5

- n_{var} = *percentage of individuals in the group with* $CV_T > CV_S$
- CV_{P50} = *median of intra-individual coefficient of variation*
- CV_{P90} = *90 percentile of intra-individual coefficient of variation*
- d_{K50} = *critical difference based on* CV_{P50}
- d_{K90} = *critical difference based on* CV_{P90}
- CV_T = *total coefficient of variation of one individual from a reference population*
- CV_S = *intra-run coefficient of variation*

Using the Mann-Whitney test (10) we have investigated for which parameters there were significant differences, between males and females, between male smokers and non smokers, between female smokers and non smokers and between female with and without oral contraceptives. For the difference between males and females only the lymfocyte count seems to be of relevance *(table 2.3.4.)*. In the male smokers/non smokers group there are relevant differences, for the PDW and the MPV, especially in the CV_{P90} and d_{K90}. The intra-individual variations of female smokers and non smokers only differed in the leucocyte counts for CV_{P50}, d_{K50}, CV_{P90} and d_{K90} *(table 2.3.5.)*.

Table 2.3.4.
Significant differences in the intra-individual variations during one day between males and females ($p < 0.05$)

male/female	n	CV_S %	CV_{P50} %	d_{K50} %	CV_{P90} %	d_{K90} %
Lymphocytes						
– males	23	1.8	7.4	21.5	26.4	74.8
– females	39	1.8	10.8	31.0	24.5	69.5
MPV						
– males	23	3.0	0.0	8.5	3.5	13.0
– females	39	3.0	0.0	8.5	3.8	13.7

– *n = number of persons*

Table 2.3.5.
Significant differences in the intra-individual variations during one day between smokers and non smokers ($P < 0.05$)

male	n	CV_S %	CV_{P50} %	d_{K50} %	CV_{P90} %	d_{K90} %
PDW						
– smokers	7	3.4	0.0	9.6	3.0	12.8
– non smokers	16	3.4	0.0	9.6	18.6	53.5
MPV						
– smokers	7	3.0	0.0	8.5	5.0	16.5
– non smokers	16	3.0	0.0	8.5	1.0	8.9
female						
Leucocytes						
– smokers	8	7.0	3.1	21.7	12.5	40.5
– non smokers	31	7.0	10.3	35.2	19.9	60.0

In *table 2.3.6.* are summarised the critical differences based on s_L (d_{K50} and d_{K90}) for both males and females, expressed in absolute values.

Table 2.3.6.
The critical difference observed during one day based on s_L

Parameter	*Dimension*	*Mean values*		d_{K50}		d_{K90}		*Transverse reference values*	
		male	*female*	*male*	*female*	*male*	*female*	*male*	*female*
Erythrocytic parameters									
Erythrocytes	T/l	4.88	4.55	0.53	0.50	0.80	0.75	4.2-5.6	3.7-5.0
Haemoglobin	mmol/l	9.4	8.6	1.0	0.9	1.5	1.4	8.5-11.0	7.5-10.0
Haematocrit	l/l	0.45	0.43	0.05	0.05	0.08	0.07	0.41-0.51	0.36-0.47
Red cell Distribution Width	%	14.6	14.6	0.9	0.9	1.1	1.1	14.3-17.6	14.3-17.6
Thrombocytic parameters									
Platelets	G/l	236	257	54	59	70	76	171-303	194-358
Mean Platelet Volume	μm^3	9.8	9.8	1.1	1.1	1.5	1.5	8.0-9.9	8.0-9.9
Platelet Distribution Width	%	38.4	38.4	4.4	4.4	6.0	6.0	33.8-42.1	33.8-42.1
Plateletcrit	l/l	0.25	0.25	0.05	0.05	0.11	0.11	0.15-0.35	0.15-0.25
Leucocyte and Differential Count									
Total leucocytes	G/l	6.8	6.8	2.4	2.4	4.1	4.1	5-10	5-10
Neutrophils	%	59	59	11	11	25	25	40-75	40-75
Lymphocytes	%	31	31	9	9	21	21	15-50	15-50
Monocytes	%	4.8	4.8	1.9	1.9	3.5	3.5	2-8	2-8
Eosinophils	%	3.0	3.0	1.6	1.6	2.6	2.6	0-5	0-5
Basophils	%	0.9	0.9	0.6	0.6	1.1	1.1	0-2	0-2
Large Unstained Cells	%	0.9	0.9	0.5	0.5	0.9	0.9	<2.2	<2.2
High Peroxidase Cells	%	0.6	0.6	0.5	0.5	0.9	0.9	<2.5	<2.5

– mean values are taken over all time points and all individuals

References

1. **Winkel P., Statland B.E., Saunders A.M., Osborn H. and Kupperman H.**
   Am. J. Clin. Pathol. 1981; 75: 693-700

2. **Mansberg H.P., Saunders A.M. and Groner W.**
   J. Histochem. Cytochem. 1974; 22: 711-724

3. **Statland B.E., Winkel P., Harris S.C., Burdsall M.J and Saunder A.M.**
   Am. J. Clin. Pathol. 1978; 69: 48-54

4. **Statland B.E. and Winkel P.**
   CRC Crit. Rev. Clin. Lab. Sci. 1977; 10: 105-144

5. **Stamm D.**
   Control of Analytical Variation in the Production of ReferenceValues. In: Gräsbeck R. and Alström T, ed. Reference Values in Laboratory Medicine. New York: John Wiley & Sons, 1981; 109-126

6. **Young D.S.**
   Clin. Chem. 1978; 24: 212-222

7. **Rümke C.C., Bezemer P.D. and Kuik D.J.**
   J. Chron. Dis. 1975; 28: 661-668

8. **Costongs G.M.P.J., Janson P.C.W., Bas B.M., Hermans J., van Wersch J.W.J. and Brombacher P.J.**
   J. Clin. Chem. Clin. Biochem. submitted for publication 1984

9. **Friedman M.**
   J. Am.. Statist. Assoc. 1937; 32: 675-701

10. **Armitage P.**
    Statistical Methods in Medical Research. Oxford: Blackwell Sc Publications, 1971.

2.4. Coagulation parameters

Introduction

In the difficult study of pathological processes concerned with blood coagulation, proper interpretation of laboratory parameters is important.
Normally a comparison is made with the so called reference values: which are determined from blood samples from an apparently healthy population. The individual data of a patient are compared to some characteristics like percentiles determined in this reference group. This comparison concentrated on inter-individual variation. Usually the patient's results are called "pathological" when they are outside the range of the so called reference values (1,2,3). An other mode of interpretation of patient's data is needed for repeated or longitudinal observations. They ask for comparison, with intra-individual variation.

Reference values are associated with different sources of variability, one of them being the analytical techniques, which play an important role in coagulation determinations. Improvement of analytical techniques and procedures during the last years have enabled us to obtain higher degrees of accuracy and precision. Another source of variability is the biological variation within one day. The present paper is concerned with these questions. To study variance components we took at fixed times of one day (i.e. 8.30 and 11.00 a.m. and 2.00 and 4.30 p.m.) blood specimens from one individual in order to assess the intra-individual variations (CV_P) of coagulation parameters during that day.

The total variation within one day (CV_T) is a result of biological (CV_B) analytical (CV_A) and "other" influences (CV_O) (4,6)
In order to differentiate between physiological and pathological deviations the critical differences of each parameter during one day should be known. In this study a number of 11 blood coagulation parameters have been investigeted in a group of 60 healthy volunteers in the age of 18 to 57 years (23 males and 37 females).

Assumming the analytical variation components to be known, the biological one's were computed. Moreover, critical differences have been determined (5,6,7)

Results and discussion

In order to investigate whether some systematic pattern existed in the consecutive parameter values during the day the Friedman rank test (8) was applied. No such patterns have been observed, except slight trends for fibrinogen (increase during the day) and for the activated partial thromboplastin time (decrease during the day). Plasminogen shows a significant higher Friedman rank values at 8.30 a.m.

In blood samples taken at different times of one day, intra-individual variations, only due to the total physiological variability were calculated. Two situations can occure either $CV_T > CV_S$, in which case CV_P is calculated, or $CV_T \leq CV_S$ in which case CV_P is set to zero. In *table 2.4.1* all results are given including n_{var} (percentage of individual in the group in whom $CV_T > CV_S$). In four tests (antithrombin III, α_2-antiplasmin, plasminogen and factor X) considerable biological variability in more than 85% of participating individuals was found ($n_{var} > 85\%$). Intermediate variability (n_{var}: 51 - 85%) was seen in three tests (calcium

thromboplastin time, activated partial thromboplastin time and fibrinogen), while least variability was found (n_{var} <50%) in four other tests (normotest, thrombotest, factor V and thrombin time).

The within-day intra-individual variation CV_{P50} and CV_{P90} were greater than zero for the plasma constituents except for the CV_{P50} of normotest, thrombotest, thrombin time and factor V, CV_{P90} of thrombotest was also smaller than CV_S.
As shown in *table 2.4.1* the intra-individual variations varied from 0.0 - 6.6% (CV_{P50}) and from 0.0 - 16.4% (CV_{P90}). The critical differences varied from 9.9 - 19.5% (d_{K50}) and from 14.8 - 46.7% (d_{K90}).

Table 2.4.1.
Intra-individual variation during one day

Analyte	n_{var} %	CV_S %	CV_{P50} %	d_{K50} %	CV_{P90} %	d_{K90} %
Normotest	20	5.3	0.0	15.0	5.9	22.4
Thrombotest	3	6.3	0.0	17.8	0.0	17.8
Calcium thromboplastin time	60	3.4	0.9	9.9	7.8	24.1
Activated partial thromboplastin time	77	3.3	3.4	13.4	9.7	29.0
Thrombin time	28	5.0	0.0	14.1	4.0	18.1
Fibrinogen	75	3.1	3.5	13.2	13.0	37.8
Factor V	28	3.5	0.0	9.9	3.9	14.8
Antithrombin III	95	2.1	5.2	15.9	16.3	46.5
α_2-Antiplasmin	92	2.0	6.6	19.5	16.4	46.7
Plasminogen	92	2.0	3.8	12.1	15.4	43.9
Factor X	88	2.2	4.8	14.9	13.7	39.2

- n_{var} = *percentage of individuals with* $CV_T > CV_S$
- CV_{P50} = *median intra-individual coefficient of variation*
- CV_{P90} = *90 percentile of intra-individual coefficient of variation*
- d_{K50} = *critical difference based on* CV_{P50}
- d_{K90} = *critical difference based on* CV_{P90}
- CV_T = *total coefficient of variation of one individual from a reference population*
- CV_S = *intra-run coefficient of variation*

Applying the Mann-Whitney test (9) to the CV_P values significant differences for the intra-individual variations of plasminogen between the male and female groups have been found, which seems to be of clinical importance *(table 2.4.2.)*.
No differences in intra-individual variations are found for the other parameters in these groups.
No significant differences in intra-individual variations are found between the male smokers/ non smokers groups, the female smokers/non smokers groups and the female groups using/ not using oral contraceptives.

Table 2.4.2.
Significant differences in the intra-individual variations during one day between males and females (P <0.05)

male/female	n	CV_S %	CV_{P50} %	d_{K50} %	CV_{P90} %	d_{K90} %
plasminogen						
– males	23	2.0	3.0	10.2	11.2	32.2
– females	37	2.0	4.3	13.4	17.9	50.9

– *n = number of persons*

The use of d_{K50} or d_{K90} is arbitrary. However, for clinical decisions d_{k90} is preferable for maximum specifity, where as greater sensitivity is obtained using d_{K50} *(table 2.4.3.)*.

Table 2.4.3.
Critical diffenrence on one day based on s_L

Analyte	*S.I. units*	*mean values*	d_{K50}	d_{K90}	*transverse reference values*
Normotest	sec.	37	6	9	<41
Thrombotest	sec.	42	8	8	<44
Calcium thrombo-plastin time	sec.	15	1.7	3.7	12-16
Activated partial thromboplastin time	sec.	23	3	7	22-34
Thrombin time	sec.	19	3	4	16-22
Fibrinogen	g/l	2.3	0.3	0.9	1.7-4.0
Factor V	%	92	11	15	>70
Antithrombin III	%	105	17	49	>70
α_2-Antiplasmin	%	102	20	48	>70
Plasminogen	%	101	13	45	>70
Factor X	%	102	16	40	>60

– *mean values are taken over all time points and all individuals*

References

1. **Amador E.**
   JAMA 1975; 232: 953-955

2. **Marin H.F., Gudzinowicz B.J., Fanger H.**
   Normal Values in Clinical Chemistry: A guide to StatisticalAnalysis of Laboratory Data. New York: Marcel Dekker, 1975; 1-454

3. **Winkel P., Statland B.E.**
   Reference Values. In: Henry J›B›, ed. Clinical Diagnosis and Management by Laboratory Methods. 16th. ed. Philadelphia: W..B. Saunders, 1979; 29-52

4. **Statland B.E. and Winkel P.**
   CRC Crit. Rev. Clin. Lab. Sci. 1977; 10 105-144

5. **Young D.S.**
   Clin. Chem. 1978; 24, 212-222

6. **Stamm D.**
   Control of Analytical Variation in the Production of Reference Values. In: Grasbeck R. and Alstrom T., ed. Reference Values in Laboratory Medicine. New York: John Wiley & Sons, 1981; 109-126

7. **Costongs G.M.P.J., Janson P.C.W., Brombacher P.J.**
   J. Clin. Chem. Clin. Biochem., submitted for publication. 1984.

8. **Friedman M.**
   J. Am. Statist. Assoc. 1937; 32: 675-701

9. **Armitage P.**
   Statistical Methods in Medical Research. Oxford: Blackwell Sc. Publications, 1971.

Chapter 3

Intra-individual variations and calculated critical differences of clinical laboratory parameters during six months

3.1. Introduction

It is important in the follow up and the development of disease that a person's laboratory data at a certain moment can be compared to earlier obtained values.
For proper interpretation of changes that can be observed, knowledge on the width of occurring changes in the absence of disease is required.
In this part of our investigations the variations in 55 laboratory parameters have been recorded monthly during a period of half a year in a group of 274 healthy volunteers. The results obtained in this study have been compared to day-to-day variations in a smaller group of 16 healthy volunteers during six days.

Individuals

The investigation has been started with a group of 300 volunteers who were free of complaints concerning their health. In the first two months 21 persons withdraw their cooperation for varions reasons (inconvenience, departure from the area, etc). At the end of the six months period another five persons were removed from the study on account of apparent disease. As a result the group of individuals participating in the study consisted of 274 apparently healthy volunteers in the age of 18 to 63 years old. Some were workers in various branches of chemical industry (84 persons), others were hospital laboratory personal (82 persons) and clerical personal (28 persons), male and female nurses (63 persons) and volunteers from a variety of other groups (17 people).
The group was subdivided in 148 males and 126 females included 72 male smokers, 54 female smokers and 61 females taking oral contraceptives. Bodyweight, length and age were registered. No restrictions were imposed on diet or activity during the study. The nature and purpose of the study were explained beforehand to the individuals, and its design was approved by the Ethical Committee.

All indidivuals were judged to be healthy at the beginning of the study by medical interview; no person suffering from chronic or recurrent illness were admitted. During the study no serious illness or injury was observed apart from hematoma after venipuncture. No drugs, apart from oral contraceptives in 61 females were involved.

In a small group (n=16) an intermediate time span was investigated studying longitudinal intra-individual variations during a week period.

Materials and methods

Scheme of venipuncture:
For the day-to-day variation every day during six days 16 volunteers were subjected to venipuncture. For the month-to-month period 274 volunteers were subjected to venipuncture monthly over a period of six months. The venipuncture was carried out at the same time of the day for each volunteer.

Specimen:
All blood specimens from one individual were taken (vacuum blood collecting system) at

fixed times each month, during six months. The serum and plasma samples of all individuals were frozen at –78°C until the day of analysis, when all specimens of one individual were thawed at room temperature, mixed thoroughly and analysed in the same run for clinical chemical and coagulation parameters.
For obvious reasons haematological (cell) parameters have been determined on the day the blood was drawn.
The individuals had a 10 to 15 minutes rest in a sitting position before venipuncture. While the subject was in a comfortable sitting posture blood was collected in evacuated blood collection tubes (Terumo®).
Brief tourniquet pressure was released immediately on venipuncture.
For the clinical chemical analyses the vacuum tubes contained no additive. The analyses have been performed in serum. For haematology the tubes contained K-EDTA as an anti-coagulant and for coagulation analyses the tubes contained sodium-citrate as an anti-coagulant.

Analytical procedures:
The automated analysers used for the haematology, clinical chemistry and coagulation were the same as used in daily routine for analysis of patients' specimens *(table 2.1.1. - 2.1.2.)*.

3.2. Clinical chemical parameters

Introduction

Evaluation of serial changes in a patient's laboratory results requires an appreciation of the changes occurring in the healthy individual as a result of analytical variation and normal physiological fluctuations. More than 20 years ago R.J. Williams (1) introduced the concept of biochemical individuality and demonstrated the uniqueness of each individual in many physiological and biochemical respects.

Biological variation can be divided into an intra-individual component, reflecting longitudinal changes occurring in the same individual in time, and an inter-individual component, representing the differences between individuals.
In this study we report the intra-individual components of variance for 28 clinical chemical parameters during six months.
We also examined the effects of age, length, bodyweight, oral contraceptives, smoking and parameter levels on the intra-individual variations.

Results and discussion

Analytical variation:
The analytical within-run variations (CV_S) are presented in terms of coefficient of variation (%) in *table 3.2.1.* With the exception of the CK-MB (4.3%), iron (3.8%), ferritin (10.0%), thyroxin (6.0%) and triiodothyronine (5.7%), the CV was always less than 2.9%. Effects of long term storage of frozen specimens during 6 months at –78°C have been investigated. This has been done by analysing a number of samples directly after the blood was taken and freezing parts of these specimens. The frozen samples have been analysed after consecutive

periods of storage at –78°C (from one month up to six months). The total coefficient of variation calculated from the values from the seven samples from the same specimens with increasing storage time, was never larger than the analytical coefficient of variation. Consequently, no influence of storage at –78°C was found.

Table 3.2.1.
Intra-individual variation and the critical difference during six months (n=274)

Analyte	n_{var} %	CV_S %	CV_{P50} %	d_{K50} %	CV_{P90} %	d_{K90} %
Enzymes						
– CK	100	1.7	22.8	64.7	45.1	127.6
– CK-MB	100	4.3	31.2	89.1	54.8	155.5
– ALAT	100	0.9	30.0	84.9	47.5	134.4
– ASAT	100	0.9	12.2	34.6	25.3	71.6
– LDH	100	2.1	10.3	29.7	17.7	50.4
– α-HBDH	99	2.8	8.8	26.1	16.5	47.3
– γ-GT	100	1.3	12.9	36.7	26.2	74.2
– Alkaline phosphatase	100	0.9	7.4	21.1	14.6	41.3
– α-Amylase	100	1.2	8.7	24.8	21.8	61.8
Electrolytes						
– Sodium	67	0.6	0.5	2.2	1.3	4.0
– Potassium	100	1.0	4.5	13.0	7.3	20.8
– Chloride	86	1.0	1.3	4.6	2.4	7.4
– Calcium	97	0.8	2.3	6.9	4.2	12.1
– Phosphate	100	1.4	9.5	27.2	14.2	40.4
Intermediary metabolites						
– Bilirubin	100	1.6	18.7	53.1	28.6	81.0
– Glucose	100	1.5	12.0	34.2	20.8	59.0
– Cholesterol	100	1.8	6.5	19.1	12.3	35.2
– Triglycerides	100	2.8	21.6	61.6	34.2	97.1
– Total protein	96	1.3	2.6	8.2	5.1	14.9
– Albumin	94	1.8	3.2	10.4	6.2	18.3
– Urea	100	1.8	11.2	32.1	18.9	53.7
– Creatinine	99	1.6	5.7	16.7	13.6	38.7
– Uric Acid	100	1.0	8.0	22.8	14.3	40.5
Iron status						
– Iron	100	3.8	19.8	57.0	33.4	95.1
– Tranferrin	97	1.9	5.8	17.3	11.2	32.1
– Ferritin	82	10.0	12.8	45.9	37.8	110.6
Thyroidhormones						
– Thyroxin (T_4)	72	6.0	4.8	21.7	13.4	41.5
– Thriiodothyronine (T_3)	82	5.7	5.7	22.8	15.1	45.7

- n_{var} = *percentage of individuals in the group in whom* $CV_T > CV_S$
- CV_{P50} = *median intra-individual coefficient of variation*
- CV_{P90} = *90 percentile of intra-individual coefficient of variation*
- d_{K50} = *critical difference based on* CV_{P50}
- d_{K90} = *critical difference based on* CV_{P90}
- CV_T = *total coefficient of variation of one individual from a reference population*
- CV_S = *coefficient of variation in the run*

Day-to-day and week-to-week variaton:
We investigated month-to-month variations of serum constituents in 274 healthy individuals occurring over a period of six months. Although this time interval is probably pertinent for following the course of a chronic disease process or possibly for monitoring healthy individuals in a preventive medicine setting, the clinician is often more interested in changes occuring within an interval of two to three days or a week, especially when following the progress of the course of a disease or the response to therapeutics. Therefore, we elected to study the short term day-to-day variation of 16 healthy individuals, i.e. the biological variation occuring in a much shorter time span (day 1 versus day 2, day 1 versus day 3, day 1 versus day 4, day 1 versus day 5, and day 1 versus day 7).

In *table 3.2.2.* we compare the day-to-day and the month-to-month biological variations of seven clinical chemical parameters. These have been chosen with regard to mineral metabolism (Na,K) enzyme pattern (ALAT) and intermediary metabolism (glucose, urea, creatinine, and uric acid) (personal communication D. Stamm). All but the sodium variations of the day-to-day intra-individual variations are lower than the month-to-month intra-individual variations. So the conclusion is justified that most of the day-to-day intra-individual variations of clinical chemical parameters are smaller or similar to the month-to-month intra-individual variations.

Month-to-month intra-indivdual variation:
From the analysis results of the blood samples taken every month at the same time of the day during a period of six months, the intra-individual variations were calculated. Two situations can occur: either $CV_T > CV_S$ in which case CV_P is found positive or $CV_T \leq CV_S$ in which case CV_P can not be calculated and is set to zero. The Friedman rank test (2) was applied to every individuals' clinical chemical data. No systematic low or high values or trends were found. The results for CV_P are summarised in *table 3.2.1.*
Month-to-month intra-individual variations, CV_{P50} and CV_{P90} were all greater than zero for all serum constituents. *(table 3.2.1.).* As shown in *table 3.2.1.* critical differences (d_{K50} and d_{K90}) varied from 2.2 -89.1% (d_{K50}) and from 4.0 - 155.5% d_{K90}).

Using the Mann-Whitney test (3) we looked for significant differences in intra-individual variations between males and females. These have been found for alkaline phosphatase, calcium, glucose, cholesterol, total protein, albumin, urea, creatinine, uric acid, transferrin and ferritin. Between male smokers and non smokers no differences were found. Between female smokers and non-smokers ASAT, LDH, α-HBDH, alkaline phosphatase and albumin showed such differences. Between females with and without oral contraceptives alkaline phosphatase, α-amylase and thyroxin were different.

Table 3.2.2.
Day to day intra-individual variation for a group of sixteen healthy individuals

		Intra-individual variation					
		day to day					*month to month* ($n = 274$)
Analyte		1 vs 2 %	1 vs 3 %	1 vs 4 %	1 vs 5 %	1 vs 7 %	%
ALAT	CV_{P50}	13.5	9.2	10.6	13.3	18.6	30.0
	CV_{P90}	31.6	42.6	38.1	24.0	32.2	47.5
Sodium	CV_{P50}	0.6	0.0	1.3	0.8	0.0	0.5
	CV_{P90}	0.8	0.8	1.9	1.3	0.8	1.3
Potassium	CV_{p50}	2.6	1.3	3.1	1.4	2.2	4.5
	CV_{P90}	6.6	3.0	6.4	5.2	6.7	7.3
Glucose	CV_{P50}	6.1	3.5	8.1	6.7	7.9	12.0
	CV_{P90}	17.2	11.7	16.8	11.3	16.8	20.8
Urea	CV_{P50}	10.6	8.0	7.9	8.7	5.8	11.2
	CV_{P90}	18.8	22.7	18.9	18.9	18.7	18.9
Creatinine	CV_{P50}	2.8	2.7	2.5	2.7	2.1	5.7
	CV_{P90}	7.4	5.0	5.4	5.8	6.9	13.6
Uric acid	CV_{P50}	4.4	5.9	4.2	7.7	6.7	8.0
	CV_{P90}	12.7	10.7	9.7	12.7	12.4	14.3

In *table 3.2.3.* the differences in intra-individual variations between males and females are given. The minimum differences for the CV_{P50} and CV_{P90} respectively are 0.4% and 0.3% for calcium, the maximum differences are 8.1% and 12.5% for ferritin. In the male/female group there were relevant differences for alkaline phosphatase, creatinine and ferritin especially in the CV_{P90} and d_{k90} *(table 3.2.3.)*.

Table 3.2.3.
Significant differences in the intra-individual variation during six months between males and females ($p < 0.05$)

Analyte		n	CV_S %	CV_{P50} %	d_{K50} %	CV_{P90} %	d_{K90} %
Alkaline phosphatase	males	148	0.9	6.9	19.7	12.2	34.6
	females	126		8.2	23.3	17.8	50.4
Calcium	males	148	0.8	2.2	6.6	4.0	11.5
	females	126		2.6	7.7	4.3	12.4
Glucose	males	148	1.5	13.2	37.6	22.4	63.5
	females	126		10.4	29.7	20.1	57.0
Cholesterol	males	148	1.8	6.0	17.7	10.8	31.0
	females	126		7.2	21.0	14.1	40.2
Total protein	males	148	1.3	2.3	7.5	4.3	12.7
	females	126		3.2	9.8	5.7	16.5
Albumin	males	148	1.8	2.7	9.2	5.1	15.3
	females	126		4.2	12.9	7.0	20.4
Urea	males	148	1.8	9.7	27.9	17.8	50.6
	females	126		12.2	34.9	21.4	60.7
Creatinine	males	148	1.6	6.2	18.1	17.4	49.4
	females	126		5.3	15.7	11.7	33.4
Uric acid	males	148	1.0	7.4	21.1	13.6	38.6
	females	126		9.0	25.6	15.5	43.9
Transferrin	males	148	1.9	5.6	16.7	10.7	30.7
	females	126		6.4	18.9	12.3	35.2
Ferritin	males	148	10.0	9.0	38.1	31.4	93.2
	females	126		17.1	56.0	43.9	127.3

– *n = number of persons*

Table 3.2.4. shows the differences in intra-individual variations between female smokers/non smokers. Minimum differences were seen for albumin with a CV_{P50} of 3.8% and a CV_{P90} of 7.0%, whereas maximum differences were observed for ASAT with a CV_{P50} of 13.6% and a CV_{P90} of 26.7%. The intra-individual variations, given in *table 3.2.4*, are clinically relevant only for LDH.

Table 3.2.4.
Significant differences in the intra-individual variations during six months between female smokers/non smokers ($p < 0.05$)

Analyte		n	CV_S %	CV_{P50} %	d_{K50} %	CV_{P90} %	d_{K90} %
ASAT	smokers	54	0.9	13.6	38.6	26.7	75.6
	non smokers	72		10.3	29.2	21.5	60.9
LDH	smokers	54	2.1	11.9	34.2	20.0	56.9
	non smokers	72		9.1	26.4	13.3	38.1
α-HBDH	smokers	54	2.8	9.9	29.1	17.6	50.4
	non smokers	72		7.9	23.7	14.2	40.9
Alkaline phosphatase	smokers	54	0.9	9.3	26.4	19.0	53.8
	non smokers	72		7.5	21.4	16.5	46.7
Albumin	smokers	54	1.8	3.8	11.9	7.0	20.4
	non smokers	72		4.4	13.4	7.0	20.4

In the females using/not using oral contraceptives, there are clinically relevant differences for α-amylase and thyroxin *(table 3.2.5.)*.

Table 3.2.5.
Significant differences in the intra-individual variations during six months between females using/not using oral contraceptives ($p < 0.05$)

		n	CV_S %	CV_{P50} %	d_{K50} %	CV_{P90} %	d_{K90} %
Alkaline phosphatase	using oral contraceptives	61	0.9	9.2	26.1	19.4	54.9
	not using oral contraceptives	59		7.6	21.6	16.8	47.6
α-Amylase	using oral contraceptives	61	1.2	9.6	27.4	28.5	80.7
	not using oral contraceptives	59		8.6	24.6	16.1	45.7
Thyroxin (T_4)	using oral contraceptives	61	6.0	7.9	28.1	16.6	49.9
	not using oral contraceptives	59		4.3	20.9	10.2	33.5

In *table 3.2.6.* are summarised the critical differences (d_{k50} and d_{k90}) based on s_L for both males and females expressed in absolute values (SI units).
Examining scatter-diagrams and correlation coefficients we found no correlations between intra-individual variation and age, length, bodyweight and levels of corresponding laboratory parameters.

Table 3.2.6.
The critical differences observed during six months for males and females based on s_L

Analyte		*mean values*	d_{K50}	d_{K90}	*transverse reference values*
		S.I.	*units*		
Enzymes					
– CK	U/l	98	63	125	< 240
– CK-MB	U/l	11	10	17	< 25
– ALAT	U/l	15	13	20	< 35
– ASAT	U/l	20	7	14	< 30
– LDH	U/l	286	85	144	< 450
– α-HBDH	U/l	114	30	54	90-180
– γ-GT	U/l	22	8	16	4-50
– Alkaline phosphatase	U/l	71	15	29	50-125
– α-Amylase	U/l	122	30	75	70-300
Electrolytes					
– Sodium	mmol/l	142	3	6	133-145
– Potassium	mmol/l	4.4	0.6	0.9	3.5-5.1
– Chloride	mmol/l	103	5	8	95-107
– Calcium	mmol/l	2.4	0.2	0.3	2.2-2.6
– Phosphate	mmol/l	1.1	0.3	0.4	0.9-1.5
Intermediary metabolites					
– Bilirubin	μmol/l	8.1	4.3	6.6	3.4-17.0
– Glucose	mmol/l	4.4	1.5	2.6	3.5-5.6
– Cholesterol	mmol/l	5.4	1.0	1.9	4.0-7.5
– Triglycerides	mmol/l	1.60	0.99	1.55	0.85-2.00
– Total protein	g/l	72	6	11	65-80
– Albumin	g/l	43	4	8	35-50
– Urea	mmol/l	4.9	1.6	2.6	3.0-7.0
– Creatinine	μmol/l	85	14	33	45-130
– Uric Acid	mmol/l	0.27	0.06	0.11	0.16-0.43
Iron status					
– Iron	μmol/l	19	11	18	10-30
– Transferrin	g/l	3.2	0.6	1.0	2.0-3.7
– Ferritin	ng/ml	74	34	82	12-302
Thryroidhormones					
– Thyroxin (T_4)	nmol/l	109	24	45	60-150
– Triiodothyronine (T_3)	nmol/l	2.3	0.5	1.1	1.3-3.0

– *Mean values are taken over all months and all individuals*

References

1. **Williams R.J.**
   Biochemical Individuality. New York: John Wiley & Sons, 1956
2. **Friedman M.**
   J. Am. Statist. Assoc. 1937; 32: 675-701
3. **Armitage P.**
   Statistical Methods in Medical Research. Oxford: Blackwell Sc. Publications, 1971.

3.3. Haematological parameters

Introduction

In the previous chapter we introduced an evaluation of serial changes in a patient's labaratory results during one day (1,2,3). This study gives the results of the haematological variation occurring in apparently healthy individuals from day-to-day and month-to-month.

The introduction of an automated cell counter (Hemalog 8/90, Technicon Instruments Corporation, Tarrytown, New York), and a leucocyte differential count automated cell counter (H-6000® Technicon Instruments Corporation, Tarrytown, New York) has resulted in the achievement of much greater precision in assessing cell counts, haemoglobin concentration, haematocrit ratio and leucocyte cell types as compared with the classical methods.

Results and discussion

Analytical variation:
The analytical day-to day variations (CV_L) are presented in terms of coefficient of variation (%) in *table 3.3.1.* With the exceptions of basophils, large unstained cells and high peroxidase cells, the CV was always less than 9%. It should be noted that the analytical variance for each cell type reflects the combined effects of the variance encountered in measuring total leucocyte count and that encountered in measuring the fraction number (differential) of the particular cell type.

Day-to-day variation:
As can be seen in *table 3.3.2.* the computed day-to-day CV_{P90} values for nearly all parameters are not significantly different in comparison to the month-to-month CV_{P90}. The conclusion can be that the "long-term" CV_{P90} and CV_{P50} obtained from month-to-month can also be used for the judgement of "short-term" fluctuations in haematological laboratory values. The results shown are in good agreement – as far as comparison is possible – with the results, published by Statland et al. (6).

Month to month variation:
The Friedman rank test (4) was applied to find out if there exists any systematic low or high values or upward or a downward trends over a six months period. In our data no such systematic phenomena were found.
The variability of the haematological parameters is expressed as n_{var} (i.e. the percentage of the variable individuals per parameter).
In *table 3.3.1.* are given the computed results for CV_{P50}, CV_{P90}, d_{K50} and d_{K90}. As can be seen the CV_{P50} values vary between 0.0 and 32.0%. These results are in agreement with the results obtained by Statland et al. (7). For the d_K values, calculated from the CV_P values, no comparisons with results from literature are possible, as these have not been reported before. The d_{K50} varied from 10.2 - 102.5%, the d_{K90} from 14.2 - 164.1%.

Table 3.3.1.
Intra-individual variation and the critical difference during six months (n=274)

Analytical parameters	n_{var} %	CV_L %	CV_{P50} %	d_{K50} %	CV_{P90} %	d_{K90} %
Erythrocytic parameters						
– Erythrocytes	57	3.8	1.4	11.5	5.0	17.8
– Haemoglobin	41	3.6	0.0	10.2	4.2	15.6
– Haematocrit	34	3.7	0.0	10.5	3.4	14.2
– Red cell Distribution Width	97	2.1	3.7	12.0	5.3	16.1
Thrombocytic parameters						
– Platelets	49	8.1	0.0	22.9	10.6	37.7
– Mean Platelets Volume	89	3.8	4.3	16.2	8.0	25.1
– Platelet Distribution Width	73	4.0	3.2	14.5	6.3	21.1
– Plateletcrit	95	6.9	11.6	38.2	20.6	61.4
Leucocyte and Differential Count						
– Total leucocytes	86	7.9	8.9	33.7	17.3	53.8
– Neutrophils	98	2.0	6.3	18.7	12.5	35.8
– Lymphocytes	100	2.3	10.8	31.2	21.3	60.6
– Monocytes	97	7.8	16.4	51.4	30.7	89.6
– Eosinophils	97	8.6	20.8	63.7	41.3	119.3
– Basophils	96	11.3	32.0	96.0	56.9	164.1
– Large Unstained Cells	94	15.4	25.9	85.2	42.7	128.4
– High Peroxidase Cells	74	28.1	22.9	102.5	49.1	160.0

– n_{var} = *percentage of individuals in the group in whom* $CV_T < CV_L$
– CV_{P50} = *median intra-individual coefficient of variation*
– CV_{P90} = *90 percentile of intra-individual coefficient of variation*
– d_{K50} = *critical difference based on* CV_{P50}
– d_{K90} = *critical difference based on* CV_{P90}
– CV_T = *total coefficient of variation of one individual from a reference population*
– CV_L = *day-to-day coefficient of variation*

Table 3.3.2.
Day-to-day intra-individual variation for a group of sixteen healthy individuals

month		*Intra-individual variation*					
		day to day					*month-to-*
Analytical parameters		*1 vs 2* %	*1 vs 3* %	*1 vs 4* %	*1 vs 5* %	*1 vs 7* %	*n = 274* %
Erythrocytes parameters							
– Erythrocytes	CV_{P50}	0.0	0.0	0.0	0.0	2.1	3.0
	CV_{P90}	5.8	5.1	4.9	5.8	5.8	5.8
– Haemoglobin	CV_{P50}	0.0	0.0	0.0	0.0	0.0	2.0
	CV_{P90}	5.0	5.0	4.8	5.1	4.9	4.9
– Haematocrit	CV_{P50}	0.0	0.0	0.0	0.0	0.9	1.2
	CV_{P90}	4.4	4.1	0.0	4.0	4.1	4.1
– Red cell Distribution Width	CV_{P50}	3.5	4.0	1.8	4.0	4.0	4.0
	CV_{P90}	5.4	6.0	5.4	5.8	5.9	5.6
Thrombocytic parameters							
– Platelets	CV_{P50}	0.0	0.0	0.0	0.0	0.0	4.1
	CV_{P90}	6.5	2.9	11.4	6.9	11.5	11.6
– Mean Platelet Volume	CV_{P50}	0.0	1.8	0.0	0.0	0.0	4.9
	CV_{P90}	8.5	8.3	11.1	7.8	6.2	8.4
– Platelet Distribution Width	CV_{P50}	0.0	0.0	0.0	0.0	0.0	3.8
	CV_{P90}	6.5	4.4	2.0	6.6	6.3	6.6
– Plateletcrit	CV_{P50}	0.0	0.0	0.0	0.0	0.0	12.1
	CV_{P90}	20.7	19.7	19.8	19.5	20.4	20.9
Leucocyte and Differential Count							
– Total Leucocytes	CV_{P50}	0.0	7.1	0.0	0.0	0.0	9.6
	CV_{P90}	17.6	17.7	17.7	17.7	16.3	17.7
– Neutrophils	CV_{P50}	6.0	6.4	5.8	6.0	6.4	6.5
	CV_{P90}	12.6	12.9	12.1	12.5	12.8	12.8
– Lymphocytes	CV_{P50}	9.1	9.8	10.9	10.1	10.8	10.9
	CV_{P90}	20.9	21.7	19.9	21.3	21.4	21.5
– Monocytes	CV_{P50}	0.0	4.2	16.7	0.0	0.0	17.0
	CV_{P90}	30.8	29.8	31.1	28.9	30.7	31.0
– Eosinophils	CV_{P50}	15.1	17.7	21.1	18.9	20.6	21.4
	CV_{P90}	37.9	39.9	41.0	38.6	40.9	41.7
– Basophils	CV_{P50}	0.0	23.4	29.9	31.9	0.0	32.5
	CV_{P90}	40.8	57.0	56.7	57.2	57.7	57.2
– Large Unstained Cells	CV_{P50}	23.5	25.6	26.6	27.1	26.8	27.1
	CV_{P90}	43.3	41.9	43.1	42.1	43.4	43.4
– High Peroxidase Cells	CV_{P50}	0.0	0.0	0.0	0.0	0.0	27.8
	CV_{P90}	0.0	40.0	47.9	29.7	50.1	51.6

Using the Mann-Whitney test (5) we looked for a significant difference in the intra-individual variation between males and females. Such differences were found for haemoglobin, red cell distribution width, platelet distribution width, basophils and large unstained cells. Between male smokers and non smokers, a difference was found for high peroxydase cells. Between female smokers and non smokers no difference was found. Between females with and without oral contraceptives, such differences were seen for neutrophils and monocytes. In *table 3.3.3., 3.3.4.* and *3.3.5.* differences in intra-individual variations are given. Although the reported differences are significant from a statistical point of view, they seems to be of no importance in clinical use.

Table 3.3.3.
Significant differences in the intra-individual variations during six months between males and females ($p < 0.05$)

		n	S_L CV%	S_{P50} CV%	d_{K50} %	S_{P90} CV%	d_{K90} %
Red cell Distribution Width	males	148	2.1	3.4	11.3	5.0	15.3
	females	126	2.1	4.1	13.0	5.6	16.9
Platelet Distribution Width	males	148	4.0	3.5	15.0	6.7	22.1
	females	126	4.0	2.6	13.5	5.8	19.9
Basophils	males	148	11.3	30.3	91.5	54.4	157.2
	females	126	11.3	35.1	104.3	61.4	176.6
Large Unstained Cells	males	148	15.4	23.8	88.2	42.0	126.5
	females	126	15.4	28.3	91.1	44.4	132.9

– *n = number of persons*

Table 3.3.4.
Significant differences in the intra-individual variations during six months between female smokers and non smokers ($p < 0.05$)

		n	S_L CV%	S_{P50} CV%	d_{K50} %	S_{P90} CV%	d_{K90} %
HPX	smokers	72	28.1	14.5	89.4	49.3	160.5
	non smokers	76	28.1	29.0	114.2	51.5	165.9

Table 3.3.5.
Significant differences in the intra-individual variations during six months between females using/not using oral contraceptives ($p < 0.05$)

		n	S_L CV%	S_{P50} CV%	d_{K50} %	S_{P90} CV%	d_{K90} %
Neutrophils	using oral contraceptives	61	2.0	6.9	20.3	12.9	36.9
	not using oral contraceptives	59	2.0	4.9	15.0	10.6	30.5
Monocytes	using oral contraceptives	61	7.8	19.5	59.4	32.9	95.6
	not using oral contraceptives	59	7.8	15.4	48.8	30.3	88.5

In *table 3.3.6.* are summarised the critical differences (d_{K50} and d_{K90}) based on s_L for both males and females expressed in current values.

Table 3.3.6.
Critical differences observed during six months for males and females based on s_L

Analytical parameters	*Dimension*	*Mean values*	d_{K50}	d_{K90}	*Transverse reference values*
Erythrocytic parameters					
– Erythrocytes	T/l	4.66	0.54	0.83	3.7-5.6
– Haemoglobin	mmol/l	9.1	0.9	1.4	7.5-11.0
– Haematocrit	l/l	0.44	0.05	0.06	0.37-0.51
– Red cell Distribution Width	%	15.5	1.9	2.5	14.3-17.6
Thrombocytic parameters					
– Platelets	G/l	246	56	93	130-350
– Mean Platelet Volume	μm^3	8.8	1.4	2.2	8.0-9.9
– Platelet Distribution Width	%	41.9	6.1	8.8	33.8-42.1
– Plateletcrit	l/l	0.21	0.08	0.13	0.15-0.35
Leucocyte and Differential Count					
– Total leucocytes	G/l	6.6	2.3	3.6	5-10
– Neutrophils	%	59	11	21	40-75
– Lymphocytes	%	32	10	19	15-50
– Monocytes	%	4.7	2.4	4.2	2-8
– Eosinophils	%	3.1	2.0	3.7	0-5
– Basophils	%	0.9	0.9	1.5	0-2
– Large Unstained Cells	%	0.8	0.7	1.0	0-2.2
– High Peroxidase Cells	%	0.8	0.8	1.3	0-2.2

– mean values are taken over all months and all individuals

Examining scatter diagrams and correlation coefficients no correlations were found between intra-individual variations and age, length, bodyweight and parameter levels.

References

1. **Costongs G.M.P.J., Janson P.C.W., Bas B.M., Hermans J., van Wersch J.W.J. and Brombacher P.J.**
   J. Clin. Chem. Clin. Biochem. submitted for publication. 1984.

2. **Costongs G.M.P.J., Janson P.C.W., Bas B.M., Hermans J., Brombacher P.J. and van Wersch J.W.J.**
   J. Clin. Chem. Clin. Biochem., submitted for publication. 1984.

3. **Costongs G.M.P.J., Bas B.M., Janson P.C.W., Hermans J., Brombacher P.J. and van Wersch J.W.J.**
   Haemostasis, submitted for publication. 1984.

4. **Friedman M.**
   J. Am. Statist. Assoc. 1937; 32: 675-701

5. **Armitage P.**
   Statistical Methods in Medical Research. Oxford: Blackwell Sc. Publications; 1971.

6. **Statland B.E., Winkel P., Harris S.C., Burdsall M.J. and Saunders A.M.**
   Am. J. Clin. Pathol. 1978; 69: 48-54

7. **Statland B.E. and Winkel P.**
   CRC Crit. Rev. Clin. Lab. Sci. 1977; 10: 105-144

3.4. Coagulation parameters

Introduction

In a previous chapter we reported on various influences on short term intra-individual variations of serum, blood and plasma constituents in a group of 62 healthy volunteers.

Changes in clinical chemical, haematological and coagulation parameters over a 8 hour period of time have been studied and the effects of smoking, oral contraceptives and sex were investigated (1,2,3).
Although these variations may be relevant for the short term control of diseases, the medical specialist with his in- and outpatients and the general practitioner are generally more interested in the changes developing over longer periods, e.g. weekly and/or monthly.

In order to eliminate the influence of time of blood sampling on the laboratory values all samples were taken at one fixed time for each individual. In this study we examined the intra-individual variability of 11 coagulation parameters during six months, plasma-samples were deep frozen and all analyses were performed at the same time.

Results and discussion

Analytical variation:
The analytical within-run variation (CV_S) is presented in terms of coefficient of variation (%) in *table 3.4.1.* With the exception of the thrombotest, normotest and the thrombin time, the CV was always less than 4%. The amidolytic tests have a CV less than 2.2%. The effect of long term storage of the frozen specimens during six months at –78°C was neglectable. (par 3.2.)

Month-to-month intra-individual variation:
In blood samples taken monthly at the same time of the day during six months, the coagulation parameters were determined. From the results the intra-individual variations have been calculated. Two situations can occur either $CV_T > CV_S$ in which case positive CV_P is found or $CV_T \leq CV_S$ in which case CV_P is set to zero. The Friedman rank test (4) was applied to every individuals coagulation data. No systematic low or high values or trends were found. In table 3.4.1. all results were given including n_{var} (percentage of individuals in the group in whom $CV_T > CV_S$).

Month-to-month intra-individual variations, CV_{P50} and CV_{P90} (median and 90 percentile of intra-individual variation), were all significantly different from zero for all constituents except for the CV_{P50} of thrombotest and thrombin time *(table 3.4.1.)*, where the $CV_T < CV_S$. As shown in *table 3.4.1.* the critical differences (d_{K50} and d_{K90}) varied from 10.6 - 29.6% (d_{K50}) and from 19.6 - 54.2% (d_{K90}).

Using the Mann-Whitney test (5) on the CV_P values we have investigated for which parameters there were significant differences in intra-individual variation between males and females. Such differences were found for calcium thromboplastin time, thrombin time, factor V, antithrombin III, α_2-antiplasmin, plasminogen and factor X. Between male

Table 3.4.1.
Intra-individual variation and the critical difference during six months (n = 274)

Parameters	n_{var} %	CV_S %	CV_{P50} %	d_{K50} %	CV_{P90} %	d_{K90} %
Normotest	71	5.3	4.2	19.1	8.3	27.9
Thrombotest	18	6.3	0.0	17.8	3.2	20.0
Calcium thromboplastin time	92	3.4	5.8	19.0	11.6	34.2
Activated partial thromboplastin time	93	3.3	6.8	21.4	12.7	37.1
Thrombin time	39	5.0	0.0	14.1	5.8	21.7
Fibrinogen	100	3.1	10.0	29.6	18.9	54.2
Factor V	77	3.5	3.6	14.2	8.5	26.0
Antithrombin III	90	2.1	3.1	10.6	6.6	19.6
α_2 - Antiplasmin	97	2.0	5.8	17.4	13.4	38.3
Plasminogen	98	2.0	7.7	22.5	17.8	50.7
Factor X	98	2.2	5.9	17.8	11.8	40.0

- n_{var} = *percentage of individuals in the group whom* $CV_T > CV_S$
- CV_{P50} = *medium intra-individual coeffcient of variation*
- CV_{P90} = *90 percentile of intra-individual coefficient of variation*
- d_{K50} = *critical difference based on* CV_{P50}
- d_{K90} = *critical difference based on* CV_{P90}
- CV_T = *total coefficient of variation of one individual from a reference population*
- CV_S = *coefficient of variation in the run*

smokers and non smokers no difference was found; between female smokers and non smokers a difference was seen for α_2-antiplasmin and between females with and without oral contraceptives, plasminogen shows such a difference.
In *table 3.4.2.* differences between males and females are given. The minimum differences for the CV_{P50} and CV_{P90} are respectively 0,7% (antithrombin III) and 0,8% (α_2-antiplasmin), the maximum differences are 1.8% (calcium thromboplastin time) and 5.6% (plasminogen). In the male/female group calcium thromboplastin time and plasminogen seems to be clinically relevant, especially in the CV_{P90} and d_{K90} *(table 3.4.2.)*. In our opinion the difference in the intra-individual variations of females smokers/non smokers is not clinical important. *(table 3.4.3.)*.
In the female groups using/not using oral contraceptives the difference in intra-individual variation for plasminogen, especially in the CV_{P90} and d_{K90} *(table 3.4.4.)* seems to be clinically important.

Table 3.4.2.
Significant differences in the intra-individual variation during six months between males and females ($p < 0.05$)

		n	CV_S %	CV_{P50} %	d_{K50} %	CV_{P90} %	d_{K90} %
Calcium thromboplastin time	males	148	3.4	5.2	17.6	9.7	29.1
	females	126	3.4	7.0	22.0	13.1	38.3
Thrombin time	males	148	5.0	0.0	14.1	4.5	19.0
	females	126	5.0	1.1	14.5	7.2	24.8
Factor V	males	148	3.5	3.2	13.4	6.9	21.9
	females	126	3.5	4.2	15.5	9.0	27.3
Antithrombin III	males	148	2.1	2.9	10.1	6.1	18.2
	females	126	2.1	3.6	11.8	7.7	22.6
α_2-Antiplasmin	males	148	2.0	5.3	16.0	13.1	37.5
	females	126	2.0	6.1	18.2	13.9	39.7
Plasminogen	males	148	2.0	6.9	20.3	13.8	39.4
	females	126	2.0	8.6	25.0	19.4	55.2
Factor X	males	148	2.2	5.4	16.5	10.9	31.5
	females	126	2.2	6.4	19.1	12.4	35.6

– *n = number of persons*

Table 3.4.3.
Significant differences in the intra-individual variations during six months between female smokers and non smokers ($p < 0.05$)

female		n	CV_S %	CV_{P50} %	d_{K50} %	CV_{P90} %	d_{K90} %
α_2-Antiplasmin	smokers	54	2.0	6.8	20.1	14.0	40.0
	non smokers	72	2.0	5.8	17.4	14.0	40.0

Table 3.4.4.
Significant differences in the intra-individual variations during six months between females who using or not using oral contraceptives ($p < 0.05$)

		n	CV_S %	CV_{P50} %	d_{K50} %	CV_{P90} %	d_{K90} %
Plasminogen:	using oral contraceptives	61	2.0	11.5	33.0	21.8	61.9
	not using oral contraceptives	57	2.0	7.3	21.4	17.7	50.4

In *table 3.4.5.* are summarised the critical differences (d_{K50} and d_{K90}) based on s_L for both males and females, expressed in current values (sec., g/l and percentage). The use of d_{K50} or d_{K90} is arbitrary. However, for clinical decisions d_{K90} is preferable for maximum specifity, whereas greater sensitivity is obtained using d_{K50}.

Table 3.4.5.
The critical differences observed during six months for males and females based on s_L.

Parameter	*units*	*mean volume*	d_{K50}	d_{K90}	*transfere reference values*
Normotest	sec	35	7	10	<41
Thrombotest	sec	45	9	10	<44
Calcium thromboplastin time	sec	15	3	5	12-16
Activated partial thromboplastin time	sec	27	6	10	22-34
Thrombin time	sec	21	4	5	16-22
Fibrinogen	g/l	2.4	0.7	1.3	1.7-4.0
Factor V	%	104	16	28	>70
Antithrombin III	%	112	13	22	>70
α_2-Antiplasmin	%	107	19	41	>70
Plasminogen	%	99	23	50	>70
Factor X	%	112	21	39	>60

– mean values are taken over all months and all individuals

Examining scatter-diagrams and correlation coefficients we found no correlations between intra-individual variations and age, length, bodyweight and levels of corresponding laboratory parameters.

References

1. **Costongs G.M.P.J., Janson P.C.W., Bas B.M., Hermans J., van Wersch J.W.J. and Brombacher P.J.**
   J. Clin. Chem. Clin. Biochem. submitted for publication. 1984.

2. **Costongs G.M.P.J., Janson P.C.W., Bas B.M., Hermans J., Brombacher P.J. and van Wersch J.W.J.**
   J. Clin. Chem. Clin. Biochem. submitted for publication. 1984.

3. **Costongs G.M.P.J., Bas B.M., Janson P.C.W., Hermans J., Brombacher P.J. and van Wersch J.W.J.**
   Haemostasis, submitted for publication. 1984.

4. **Friedman M.**
   J. Am. Statist. Assoc. 1937; 32: 675 -701

5. **Armitage P.**
   Statistical Methods in Medical Research. Oxford: Blackwell Sc. Publications, 1971.

Chapter 4

General discussion and conclusions

The objective of the present study has been stated in the introduction: to assess the intra-individual variations of laboratory parameters in clinical chemistry and in haematology in a population of healthy individuals.
For this purpose we investigated three groups of volunteers for different time spans, first month-to-month changes in a group of 274 volunteers (148 males/126 females), second a group of 62 persons (23 males/39 females) for the changes within one day. We studied in these groups 28 clinical chemical parameters, 16 haematological parameters and 11 coagulation parameters. In a small group of 16 persons we checked the variation from day-to-day during 6 days in order to certify that these values were in accordance with month-to-month intra-individual variations. The results of the investigations are to some extent surprising and always very interesting.

From a study of the literature on the (intra-individual) variability of laboratory data, it soon appeared that true comparison with our data was very difficult. Almost all published investigations were concerned with groups of persons which were too small for solid statistical evaluation of intra-individual analytical data. An exception, however, was the study of Lommel and Weyer (1). They studied a large group of highly selected origin, i.e. male soldiers between 18 and 23 years of age. This of course eliminate several influences such as age, sex, etc., on the obtained data population which made their results in general applicable to a very limited extent for general practice.

Other investigators such as van Steigterhem, et al.(2) have studied the analytical, intra-individual, and inter-individual components of variance in a group of only 10 volunteers for 34 assays encompassing 22 different constituents. Replicate blood specimens were obtained four times weekly over a 4 weeks period. Nine constituents were measured with more than one instrument.
Analytical variances of more than 30% of total variance have been reported. The use of different analysers can be considered to be the main cause of the different (sometimes large) analytical variances s_A^2.
This is in contrast with our study where all analyses have been performed in such a way that s_A^2 is kept minimal.

Harris et al. (3) applied standard statistical analysis of variance on data from weekly determinations of 15 blood constituents in each of 68 normal subjects, over a 3 months period. The purpose was to isolate and estimate biological components of observed variation. The most critical problem, that of separating variation from long-term analytical deviations in each individual's data record, was resolved with the aid of concurrent analysis of a constant "pooled" serum. They stated that comparison of isolated personal variations with inter-individual variation indicate that many common blood tests could contribute to an individually distinctive blood "profile" if substantial improvements were made in analytical

precision. This is in accordance with our opinion. However, the numbers of parameters and participants in their study were smaller than in our study and the analytical variances too large.

Young et al. (4) sampled blood, under controlled conditions, from 9 healthy subjects weekly for 10 weeks.
Eighteen serum constituents were measured in duplicate in each sample on a single occasion. In this way the analytical variation was minimised. Considerable variations in mean values and standard deviations of results were observed among the subjects. Compared with a previous study (3) from their laboratory, significantly different estimates of personal variation were noted for a number of constituents. The group studied showed as great a diversity in the mean concentrations of most constituents as was seen earlier in a larger, more heterogeneous group of normal individuals. The authors concluded that certain blood parameters depend more on personal characteristics than on broad demographic factors. It can be doubted whether their conclusions have general validity because only 9 persons participated in their study.

Winkel et al. (5) evaluated the variations in some serum consituents in a group of 11 healthy young men for two selected time intervals: short term day-to-day changes over a six day period and hour-to-hour changes over two six hour periods in different weeks. Analytical variations appeared to be of considerable influence on total variations. It must be remarked that the group size (11 young men) is too small to obtain generally valid data.

Pickup et al. (6) stressed the advent of high-capacity multi-channel analysers for the estimation of long-term variability in serum constituents. By frozen storage of specimens from 37 volunteers (20 males/17 females) with subsequent analysis in a single machine run, long-term analytical variation may be eliminated, thus sharpening the estimates of intra-individual variation. The usual population based reference ranges were found to be either insensitive or irrelevant to the study of concentration changes over time in most healthy subjects.

Williams et al. (7) studied a group of 1105 individuals. Analyses were performed, in weekly blood samples over a 12 weeks period. The assumption was tested that a reference group of individuals, categorized by age and sex would gives a narrower range of variation than does a larger mixed population. Their results implied the need for individual rather population-based reference ranges, even if the latter were from persons of similar age and the same sex. Although in their study factors influencing the s_P^2 have not been considered, their conclusions are in accordance with ours.

In general, conclusions of the investigations reported have limited values and sometimes are not in accordance with our data *(table 4.1.)*.
The use of mean values instead of 50-percentile values is often not justified; Gaussian distribution often cannot be confirmed in small groups or is not found even in larger groups.

table 4.1.

Published intra-individual variations (CV_P)

Literature references	(2)	(3)	(4)	(5)	(6)	(7)	our study CV_{P50}
n	10	68	9	11	37	1105	274
Enzymes:							
CK	82.76	–	–	–	–	60.3	22.8
ALAT	57.89	–	–	26.4	–	–	30.0
ASAT	14.79	–	15.1	24.2	–	19.3	12.2
LDH	6.19	9.0	7.3	–	–	8.0	10.3
γ-GT	–	–	–	–	–	34.7	12.9
Alkaline phosphatase	6.33	–	5.8	4.8	6.4	8.5	7.4
α-Amylase	–	–	–	–	–	–	8.7
Electrolytes:							
Sodium	0.93	–	1.4	0.7	0.5	–	0.5
Potassium	4.43	5.0	4.6	4.3	6.2	–	4.5
Chloride	1.52	1.4	2.1	2.1	–	–	1.3
Calcium	1.93	1.7	1.6	1.7	1.6	2.1	2.3
Phosphate	7.40	7.5	9.6	5.8	6.8	8.2	9.5
Intermediary metabolites:							
Bilirubin	25.59	–	–	22.0	26.0	–	18.7
Glucose	–	5.6	6.5	–	–	6.5	12.0
Cholesterol	4.39	6.4	4.2	5.3	–	7.9	6.5
Triglycerides	33.33	–	–	–	–	27.3	21.6
Total protein	2.51	2.8	2.3	2.9	3.0	3.3	2.6
Urea	14.29	11.9	13.6	12.3	11.1	16.4	11.2
Creatinine	3.83	–	4.4	4.3	–	3.1	5.7
Uric acid	8.98	10.1	8.5	7.3	–	8.8	8.0
Iron status:							
Iron	–	–	–	26.6	–	–	19.8

Due to the relatively small number of participants in some of the described studies no attention has been paid to differences within one group such as male/female, smokers/non smokers, age intervals, bodyweight, and length, females taking/not taking oral contraceptives.

During the evaluation of the present studies, the question had to be answered how to apply our results in the daily use of laboratory values and how to evaluate meaningfullness of transverse reference ranges.

In agreement with other investigators (8,9) the current use of "reference values" should now be considered. The main reason for this point of view is, that statistically developed transverse reference ranges for many parameters are often much larger than the range of

physiological intra-individual variatons. Intuitively physicians do estimate intra-individual changes in laboratory values far higher than the general "normal values". An example is available: the occurence of apparent clinical hyperthyroidism combined with thyroid hormone values in plasma which are completely compatible with "normal values". The importance of intra-individual variations is one more demonstrated by the development of clinical, treatable and curable hyperthyroidism during the use of oral contraceptives. In these cases, plasma thyroidhormone values increased, but, as we observed, often within the transverse reference ranges.

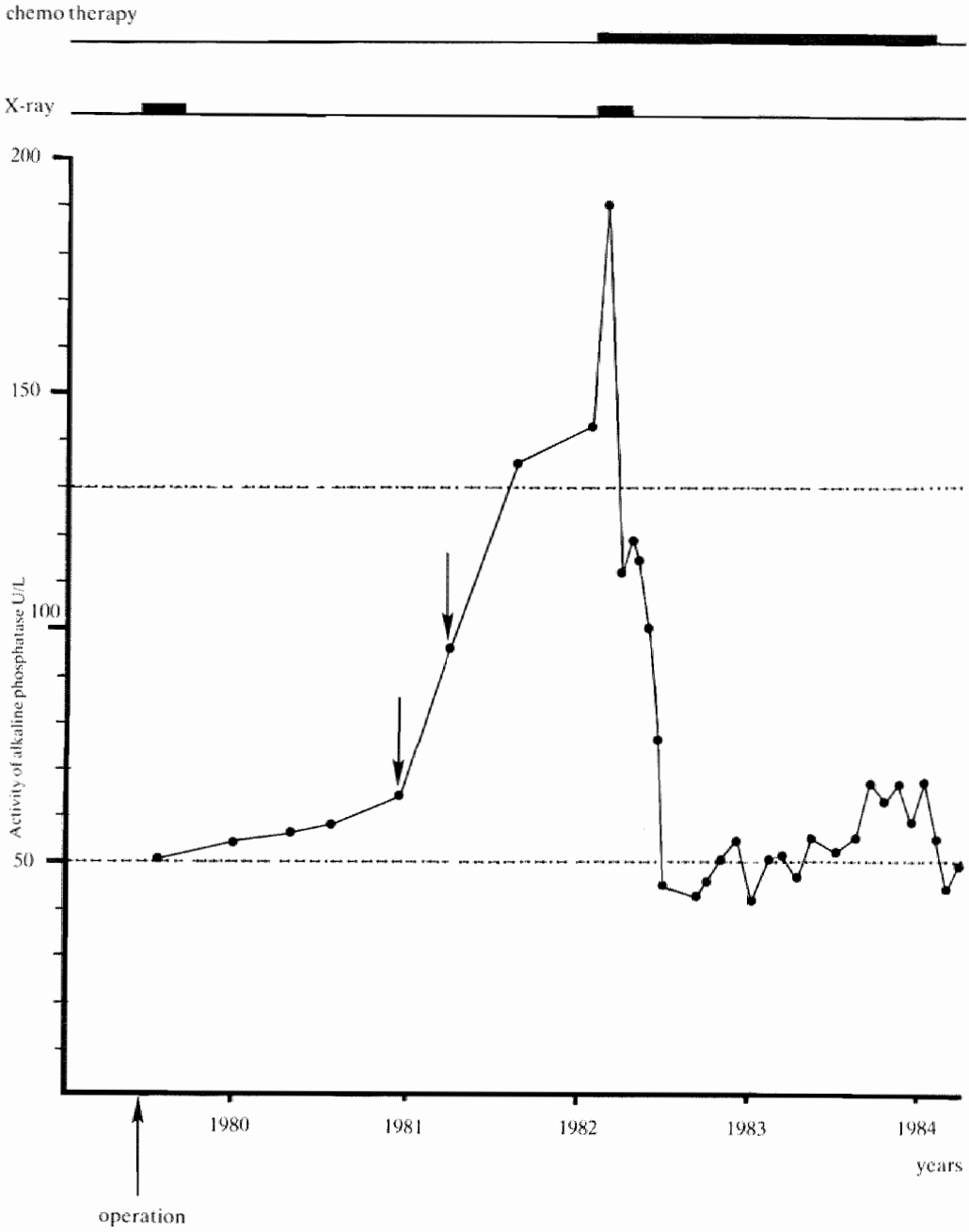
In *table 4.2.* for some examples a comparison is given of mean values, critical differences d_{K50} and d_{K90} and transverse reference values.
The importance of these values for clinical medicine is evident. Interpretation of longitudinal changes in laboratory data in one patient using critical differences, makes detection of pathological changes within the "normal reference range" possible.
A changing value of sodium from 134 to 142 mmol/l, where both values are "normal", is a pathological change. This is also true for a changing γ-glutamyl transferase values from 10 to 30 U/L although no clinician is alarmed by a single report of the last value. Another striking result of our study is the critical difference for the blood haemoglobin concentration. A change in the latter of 1.4 mmol/l, from 9.1 mmol/l to 7.7 mmol/l has a propability of being not pathological of 10%, thereas a change of 0.9 mmol/l still is propably not pathological in about 50% of cases.

table 4.2.

Comparison of some mean values with critical differences and transverse reference values.

Parameters	Units	$\bar{x}$	d_{K50}	d_{K90}	transverse reference values
γ-GT	U/l	22	8	16	4-50
Alkaline phosphatase	U/l	71	15	29	50-125
Sodium	mmol/l	142	3	6	133-145
Triglycerides	mmol/l	1.60	0.99	1.55	0.85-2.00
Creatinine	µmol/l	85	14	33	45-130
Iron	µmol/l	19	11	18	10-30
Haemoglobin	mmol/l	9.1	0.9	1.4	7.5-11.0
Platelets	G/l	246	56	93	130-350
Total leucocytes	G/l	6.6	2.3	3.6	5-10
Calcium thromboplastin time	sec	15	3	5	12-16
Activated partial thrombin time	sec	27	6	10	22-34
Antithrombin III	%	112	13	22	>70
Plasminogen	%	99	23	50	>70

From our own files we report on a patient who underwent mastectomy for mammary carcinoma. During follow up the values of alkaline phosphatase were determined at regular intervals in order to detect metastases as soon as possible. No metastases were present at the time of the operation; alkaline phosphatase was then 51 U/l (see figure). After 18 months the alkaline phosphatase value was 64 U/l and 3 months later 95 U/l. No metastases could be found with X-ray examination.



Because the changing values of the alkaline phosphatase were within the usual transverse reference range and on account of negative X-ray information, no therapy was started at that moment. After another 5 months the alkaline phosphatase value increased to the upper limit of the reference range and 6 months later it was 140 U/l. Another X-ray investigation was then performed and bone metastases were observed. Chemotherapy was started and was succesfull. After another 4 months alkaline phosphatase values had come down to 50 U/l and stayed there for the next 2 years.
On retrospective consideration we think that chemotherapy could have begun 11 months earlier, i.e. at the month when alkaline phosphatase values around 95 U/l were measured.
The critical differences for the alkaline phosphatase for this patient could be calculated:
d_{K50} (21.1%) = 11 U/l and d_{K90} (41.8%) = 21 U/l.
The change from 51 U/l to 64 U/l thus was within the critical difference d_{K90} (although exceeding the d_{K50}), the change from 51 U/l to 95 U/l evidently exceeded the critical difference d_{K90}.
All participants in the study and treatment of this case agreed that earlier treatment would have been for the benefit of this patient. Alas, critical differences were no common knowledge at that time.

It can be concluded that the use of general transverse reference ranges is primarily suitable to detect gross deviations and pathology.
The use of intra-individual variations and the calculated critical differences improves the value of laboratory data for diagnostic and clinical purpose. Our opinion is in agreement with others (1,9) regarding the necessity of considering the individual as his own reference and the use of critical difference values in longitudinal studies (chapters 1 - 3 of this thesis). It can be remarked that in screening for early detection of disease new decision limits must be determined which are different from the traditional transverse reference ranges (11).

Another question is to what extent extra information was obtained by the results of our investigations. Before being able to answer this, however, it became clear that many precautions had to be taken when determining reference values. It has been demonstrated that strict standardisation of circumstances, both pre-analytical and analytical, is absolutely necessary. An example is given by Wisser (11) regarding the plasma total protein content. The determination of reference ranges between clinical and out-patient groups leads to differences of up to 8%. This same difference, however, in intra-individual changes, may be very important and pathognomonic.

Also from our study the importance of standardised conditions for taking bloodspecimen is stressed, e.g. for the serum triglyceride concentration values. The transverse reference values in general use have been determined after 16 hours fasting. In our study the volunteers have not been fasting and the critical differences therefore are much too large to use in combination with the given transverse reference values.

The changing attitude towards the importance of reference values and their physiological variations is also seen in the recent literature.
However, only few authors paid due attention to the pre-analytical conditions, such as blood-taking equipment and "other" factors. We found a greater importance of these circumstances especially in those parameters where the analytical imprecision is very small

(most clinical chemical determinations). It is not unexpected that in determinations with greater analytical imprecision (such as the majority of haematological investigations) the pre-analytical influences are less pronounced. However, with small analytical variation, as by the introduction of highly sophisticated analysers in this field (e.g. Technicon H 6000®) the importance of pre-analytical standardisation might increase. Of course, in normal practice it is often necessary to compromise.

Conclusions:

1. The pre-analytical circumstances must be standardised:
   - adequate blood-taking equipment, i.e. preferably a standardised (vacuum) system for the taking of venous blood is necessary. Capillary blood samples should be avoided if possible.
   - patients should always be in the same position, e.g. supine
   - blood samples should always be taken at the same time of the day.
   - adequate care should be taken of the blood samples, such as storage adequate centrifugation, etc.
2. When reporting laboratory values, not only the analytical data concerning the patients' sample should be given, but also the reference values used and the critical differences. The latter are absolutely necessary for longitudinal studies.
3. As far as necessary circumstances such as ambulant or clinical state of fasting, pregnancy, use of drugs, presence of stress, and other circumstances should to be taken into account and reported.
4. It goes without saying that careful registration of the patient's history (including all laboratory values) is necessary.
5. **In longitudinal studies the transverse reference values are of little use. In contrast, the critical differences for the reported parameters are very important. It is necessary to consult the laboratory staff often in order to cope adequately with the complexity of the consideration of laboratory values.**

References

1. **Lommel H. and Weyer M.G.**
   Long I. Mainz: Verlag Kircheim & Co., 1981; 1-487
2. **van Steigterhem A.C., Robertson E.A. and Young D.S.**
   Clin. Chem. 1978; 24: 212-222
3. **Harris E.K., Kanofsky P, Shakarji G. and Cotlove E.V.**
   Clin. Chem. 1970; 16: 1022-1027
4. **Young D.S., Harris E.K. and Cotlove E.V.**
   Clin. Chem. 1971; 17: 403-410
5. **Winkel P., Statland B.E. and Bokelund H.**
   Clin. Chem. 1974; 20: 1520-1527
6. **Pickup J.F., Harris E.K., Kearns M. and Brown S.S.**
   Clin. Chem. 1977; 23: 842-850
7. **Williams G.Z., Widdowson G.M. and Penton J.**
   Clin. Chem. 1978; 24: 313-320
8. **Keller H.**
   Einflüsse auf Klinisch-chemische Messgrössen. In: Lang H., Rick W., and Büttner H., ed. Validität klinisch-chemischer Befunde. Berlin/Heidelberg/New York: Springer-Verlag, 1980; 25-49
9. **Feinstein A.R.**
   Clinical Biostatistics. St. Louis: Mosby Comp., 1977
10. **Wisser H.**
    Diskussion. In: Lang H., Rick W. and Büttner H., ed. Validität klinisch-chemischer Befunde. Berlin/Heidelberg/New York: Springer-Verlag, 1980; 51-57
11. **Haeckel R.**
    Deutsches Ärzteblatt 1979; 39: 713

Summary

In the previous chapters we described a study on longitudinal variations of laboratory parameters. In the introduction (chapter 1) the aim of the study has been defined: to assess limits of physiological longitudinal variations in one individual of clinical chemical, haematological and coagulation laboratory values in order to facilitate the diagnosis and follow-up of disease.

Statistical procedures have been introduced and explained. In chapter two an investigation has been described on the longitudinal intra-individual variaton of values of laboratory parameters during one day. Samples were collected by venipuncture at 8.30 and 11.00 a.m. and 2.00 and 4.30 p.m. on the same day from a population of 23 healthy males and 39 healthy females.
For each individual the variance of blood values has been determined. A number of 28 clinical chemical, 16 haematological and 11 coagulation parameters have been measured in all samples.
Intra-individual longitudinal variances and critical differences could be calculated and the influences of non-analytical factors such as sex, smoking, oral contraceptives, bodyweight, length and parameter value level have been studied. Moreover it has been investigated whether systematic patterns existed in the consecutive parameter values in the course of one day.

In chapter three a description has been given of a similar investigation concerning consecutive analyses of once monthly taken blood samples in a standardised scheme during a period of six months. Again all mentioned parameters have been determined and longitudinal intra-individual variations and critical differences were calculated. In this study a number of 274 healthy volunteers (148 males/126 females) participated. The influences of the same external factors as mentioned before have been investigated.
In order to bridge the gap between the within-one-day and the six-month investigation, a similar study on a smaller scale (16 participants, 23 parameters) was performed on daily blood samples during a period of six days. The results are equally reported in this chapter.

In both studies, within-one-day and over-six-months periods and in the smaller six-day study, no explicit bioligical rhythms for any parameter were found for the groups. However, in single individuals, such rhythms were often seen. It appeared that individual variations in laboratory values of one person provide far more information in diagnostic and follow-up procedures then could be obtained from the hitherto generally used transverse reference values. The great importance of the critical differences was established.

In chapter four the results from preceding chapters have been considered together and the implications for the use of intra-individual longitudinal variations and critical differences in clinical practice have been elucidated.

It could be concluded, that critical differences play a dominant role in the consideration of laboratory values, especially in the early detection and follow-up of disease. Frequent consultation of laboratory staff by physicians is necessary for optimal interpretation of blood chemistry, haematology and coagulation data.

Samenvatting

In de voorafgaande hoofdstukken is een onderzoek over longitudinale variaties van laboratoriumparameters beschreven. Het doel van deze studie (hoofdstuk 1) was om bij gezonde vrijwilligers vast te stellen hoe groot de intra-individuele variaties waren, en de kritische verschillen (critical differences) te berekenen van klinisch-chemische, haematologische en stollingsbepalingen, gedurende één dag, van dag-tot-dag en van maand-tot-maand.

In het kader van vroegtijdige herkenning en mogelijke preventie van ziekten dient men rekening te houden met de intra-individuele variaties en de analytische variaties, en de hieruit berekende kritische verschillen. De hiervoor noodzakelijke statistische werkwijzen werden gegeven.

In hoofdstuk 2 werd het onderzoek beschreven over de intra-individuele variaties van laboratoriumbepalingen gedurende een dag.
Bloed werd met behulp van een vacuum afname systeem bij 62 gezonde vrijwilligers (23 mannen/ 39 vrouwen) op een en dezelfde dag op de volgende tijdstippen afgenomen: 8.30 u, 11.00 u, 14.00 u en 16.30 uur. In de bloedmonsters werden 28 klinisch-chemische, 16 haematologische en 11 stollingsparameters bepaald.
Intra-individuele variaties en kritische verschillen werden berekend. Tevens werd nagegaan hoe de invloed was van geslacht, roken, orale contraceptiva, lichaamsgewicht, lichaamslengte en het gemeten niveau van de laboratoriumwaarden op de intra-individuele variaties.
Verder werd onderzocht of er een systematisch patroon bestond voor de diverse parameters gedurende één dag.

In hoofdstuk 3 werd een vergelijkbaar onderzoek beschreven met betrekking tot de intra-individuele variaties over een periode van 6 maanden. Eén keer per maand werden bloedmonsters afgenomen bij 274 gezonde vrijwilliggers (148 mannen en 126 vrouwen) onder standaard condities. De intra-individuele variaties van de parameters werden bepaald en de kritische verschillen berekend.
Tevens werd nagegaan bij een kleinere groep vrijwilliggers (16 personen), voor 23 parameters hoe groot de intra-individuele variatie was gedurende 6 opeenvolgende dagen. De resultaten waren vergelijkbaar met die, welke werden verkregen in het maand tot maand onderzoek.

In geen van de onderzochte perioden werden duidelijke biologische ritmes voor enige parameters gevonden. Deze werden vaak wel intra-individueel waargenomen.
Gebleken is dat longitudinale veranderingen van laboratoriumwaarden voor één bepaalde persoon wezenlijk meer bijdragen aan het inzicht in de kennis en de diagnose en/of het ziekteverloop, dat kan worden verkregen door gebruik van de algemene (transversale) referentiewaarden. Het grote belang van de zogenaamde kritische verschillen kon worden aangetoond.

In hoofdstuk 4 werden de resultaten van de voorafgaande onderzoekingen nader beschouwd en werd het belang daarvan voor het dagelijks gebruik in de geneeskunde nader toegelicht.

De voornaamste conclusie uit het beschreven onderzoek is dat de kritische verschillen, zoals die per parameter werden bepaald, van uitzonderlijk belang zijn voor een goed gebruik van laboratoriumgegevens. Dit geldt speciaal bij vroegtijdige herkenning van ziekte en bij het vervolgen van een ziekteverloop.
Ook is nogmaals gebleken dat optimaal gebruik van laboratoriumgegevens in de geneeskunde nauwe samenwerking tussen behandelende artsen en specialisten op het gebied van de laboratoriumdiagnostiek vereist.

Curriculum Vitae:

Op verzoek van de faculteit volgen hier enkele persoonlijke gegevens. Ik ben geboren op 17 december 1955 te Maastricht. Na het eindexamen Atheneum-B in 1975 aan het Henric van Veldeke College te Maastricht, liet ik mij inschrijven aan de Universiteit van Amsterdam. In november 1981 behaalde ik het doctoraalexamen farmacie met hoofdvak farmaco-therapie en bijvak biofarmacie.
Sinds december 1981 ben ik in opleiding tot klinisch chemicus bij dr. P.C.W. Janson op het klinisch chemisch laboratorium van het ziekenhuis "De Goddelijke Voorzienigheid", te Sittard alwaar dit proefschrift werd bewerkt.

G.M.P.J. Costongs

Abbreviations and definitions.

- *Longitudinal assessment:*
  Comparison of an analytical result from a patient with earlier results from the same patient

- *Transverse assessment:*
  Comparison of an analytical result from a patient with reference values obtained from a suitable reference sample group

- *Critical difference:*
  Indicates the range which covers the great majority (95%) of the differences between two obtained values of one laboratory parameter in one individual.

s_T^2 = total variance of one individual from a reference group
s_B^2 = biological variance
s_P^2 = intra-individual variance
s_I^2 = inter-individual variance
s_A^2 = total analytical variance
s_S^2 = analytical variance within one run
s_L^2 = analytical variance between runs
s_O^2 = "other" variance, e.g. specimen collection

CV_T = total coefficient of variation of one individual from a reference group
CV_B = biological coefficient of variation
CV_P = intra-individual coefficient of variation
CV_I = inter-individual coefficient of variation
CV_A = total-analytical coefficient of variation
CV_S = analytical within one run coefficient of variation
CV_L = run-to-run or long-term coefficient of variation
CV_O = "other" coefficient of variation

n_{var} = the percentage of the variable indidividuals per parameter in whom $CV_T > CV_S$